FCM, Cytotaxonomie, karyologie, cytogenetika

S využitím prezentací Honzy Sudy

P ř e h l e d

- počet chromozomů
- morfologie chromozomů
- velikost chromozómů (velikost genomu)
- barvitelnost chromozomů
- „chování“ chromozomů
- molekulární cytogenetika

Počet chromozomů

- 1882: Strassburger - *Lilium* 2n = 24
- dodnes - ca 25% krytosemenných
- nerovnoměrné pokrytí
- chromozomové atlasy (W^3TROPICOS, Taxon, CCDB database)
- požadavky:
  - přírodní materiál
  - herbářová položka
  - opakování (buňky, jedinci)

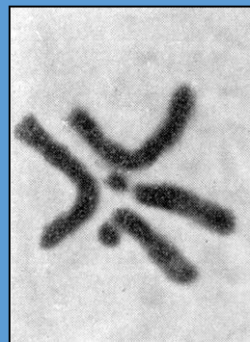
  ! somatické polyploidie, mozaiky

S u p e r l a t i v y

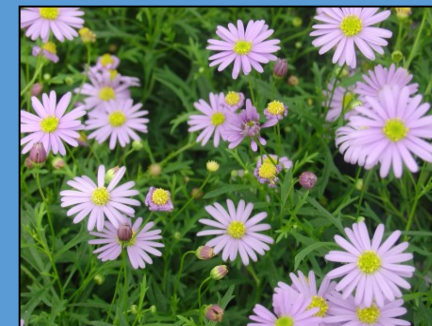
2n = 4

Spirogyra

Porphyra



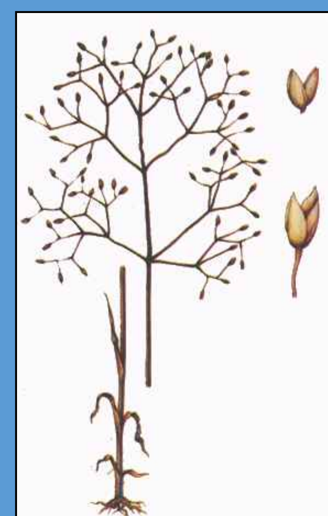
Haplopappus gracilis



Brachyscome dichrosomatica

Viola modesta

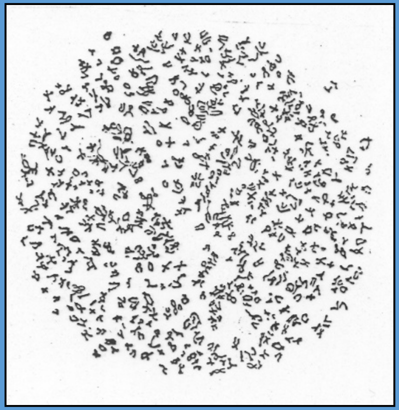
Ornithogalum tenuifolium



Zingeria biebersteiniana

Colpodium versicolor

S u p e r l a t i v y



Ophioglossum reticulatum

2n = 96x = 1440

Sedum suaveolens

2n = 80x = 640

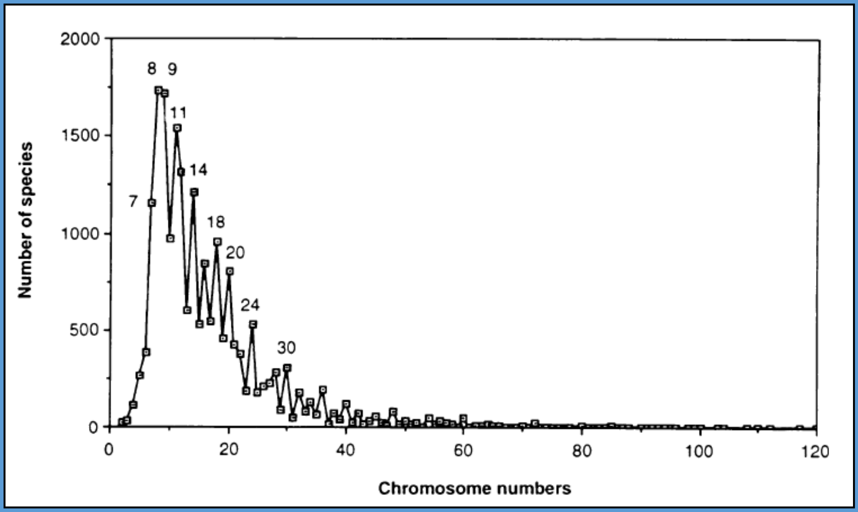
Gymnodinium

2n = c. 1028

Voanioala gerardii

2n = 50x = ca. 600

Základní chromozomové číslo



- *x*
- relativní (potíže stanovení)
- stabilní x variabilní
- změny:

  - ztráta chromozómů (*Crepis, Vicia*), fúze chromozómů (*Cardamine*)
  - Senecio – 2n = 40 (2x or 4x?)
  - (hypo)aneuploidie (*Artemisia*)
  - dibasická polyploidie (*Brassica, Maloideae*)

Základní chromozomové číslo

- *Poaceae* - vymezení podčeledí
  - *Bambusoideae*, x = 12
  - *Arundinoideae*, x = 9, 12
  - *Chloridoideae*, x = 9, 10
  - *Panicoideae*, x = 5, 9, 10
  - *Pooideae*, x = 7

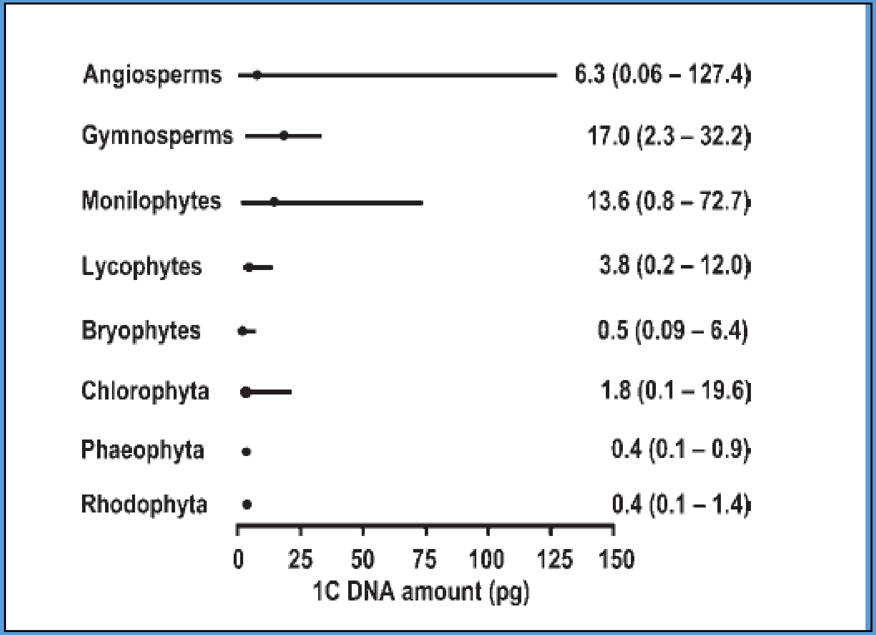
- podpora evolučních linií
  - *Caryophyllidae*, x = 9 (ostatní x = 7)

Počet chromozómů

- vymezení druhů / poddruhů
- kryptická speciace
- zdroje problémů (polyploidní komplexy)
- hybridizace
- predikce (reprodukční způsob)

Velikost genomu

- mezidruhová variabilita
- vnitrodruhová variabilita



Paris japonica

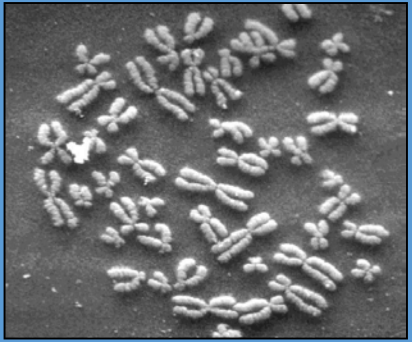
1C = 152,2 pg

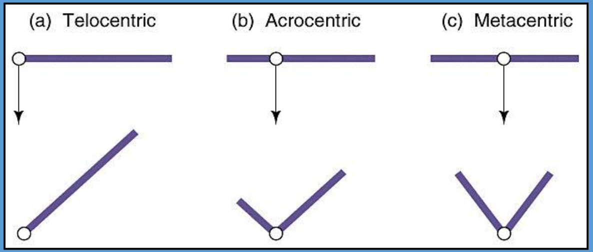
Genlisea aurea

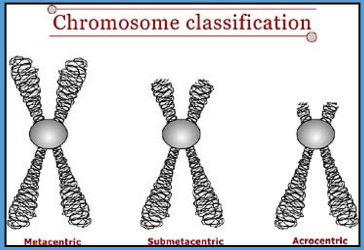
1C = 0,065 pg

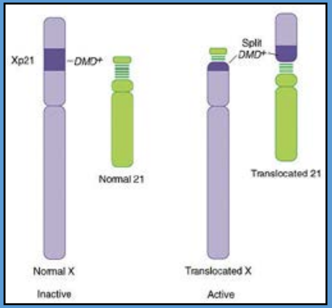
Morfologie chromozómů

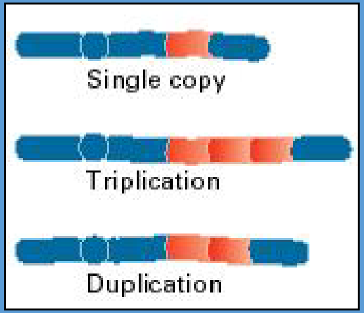
- **centromera, sekundární konstrikce, satelit**
- **difúzní centromera**
- **změny:**
  - pericentrické inverze
  - nerovnoměrné translokace
  - delece, duplikace
  - fúze

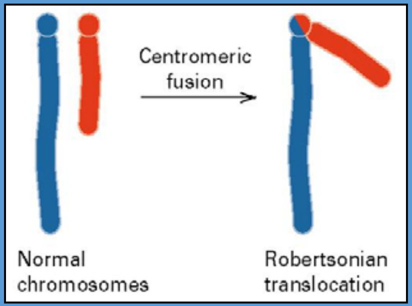








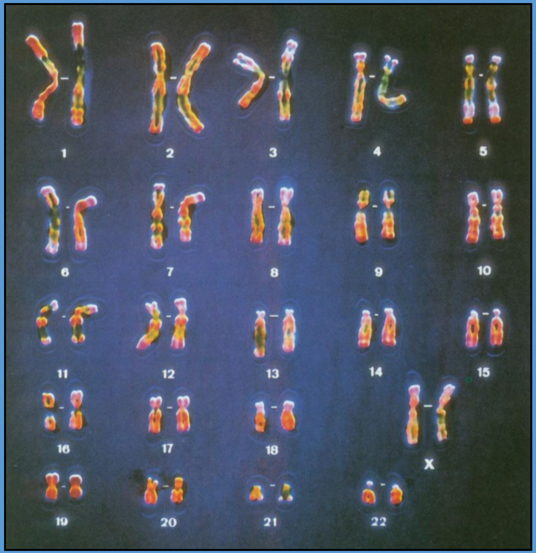




K a r y o t y p

- **symetrický x asymetrický**
- **bimodální**

- **evoluční vztahy (*Ranunculaceae* s.l.)**
- **hledání rodičů (*Fallopia, Triticum*)**

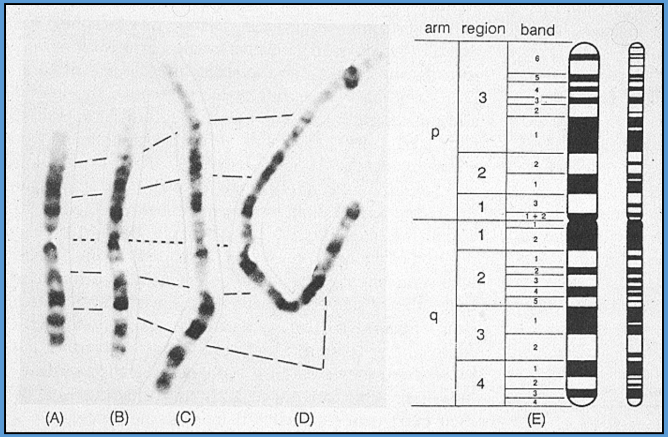


Barvitelnost chromozómů

- euchromatin x heterochromatin
- pohlavní chromozomy

- tradiční barviva (karmin, orcein, fuchsin)
- „proužkování“
  - Q-banding (quinacrin)
  - C/G-banding (Giemsa)
  - AT/GC selektivní barviva

- CCP – comparative chromosome painting



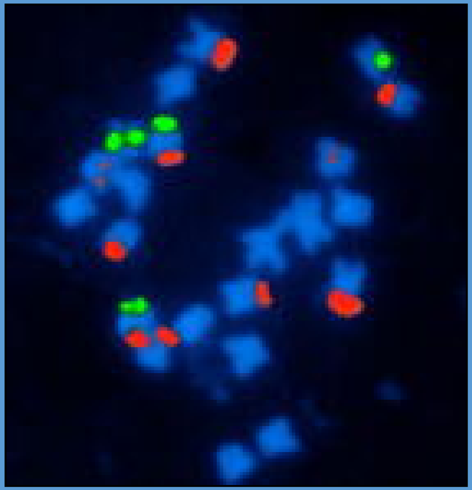
Barvitelnost chromozómů

- *In situ* hybridization, FISH, GISH

FISH – lokalizace značené DNA na chromozomech

GISH – determinuje mezidruhovou distribuci opakujících se sekvencí na chromozomech

- hledání rodičů
- původ polyploidů
- hybridizace a introgrese



Chování chromozómů

- párování v meióze
- homologní x homoeologní chromozomy
- bivalenty x polyvalenty

- typ polyploidů (auto x allo)
- evoluční příbuznost
- hybridizace a introgrese

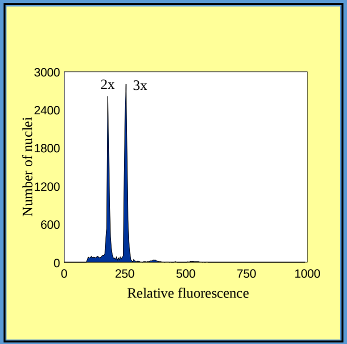
What is flow cytometry?

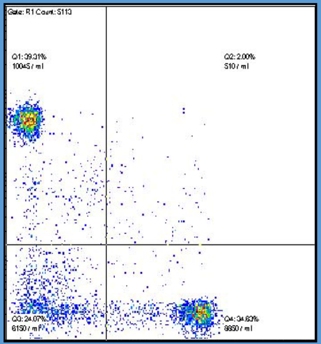
- optical characteristics of isolated particles
  (fluorescence intensity, light scatter)

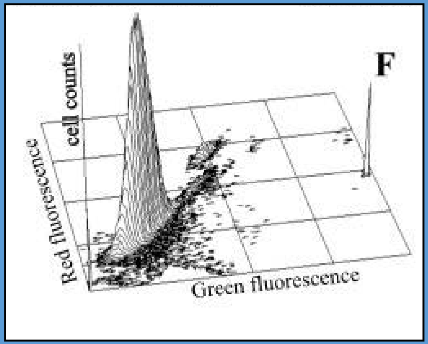
↓

- quantification of (any) chemical compound

- most common: amount of nuclear DNA (genome size)
  absolute / relative units

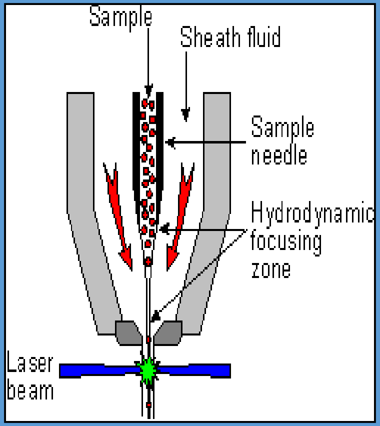


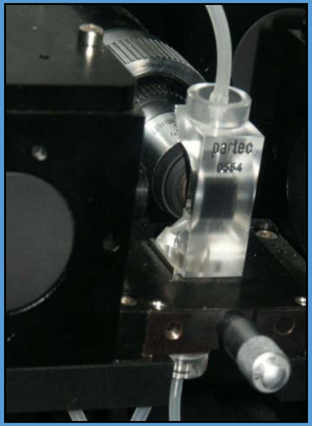


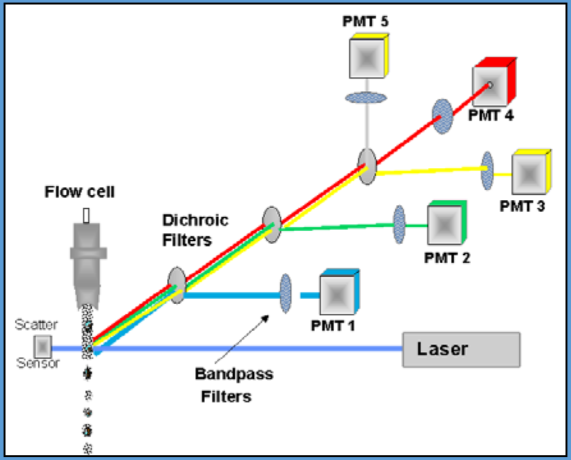


Instrumentation

- light source (laser, UV lamp, diode)
- flow chamber + fluidic system
- optical assembly (filters, mirrors)
- signal processing part (PMT, convertors)
- computer part



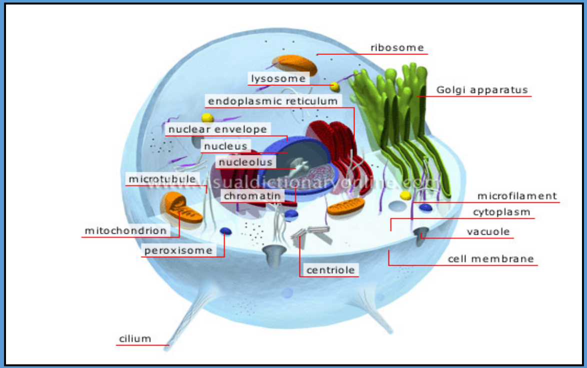




Challenges with plant material

PLANT CELLS

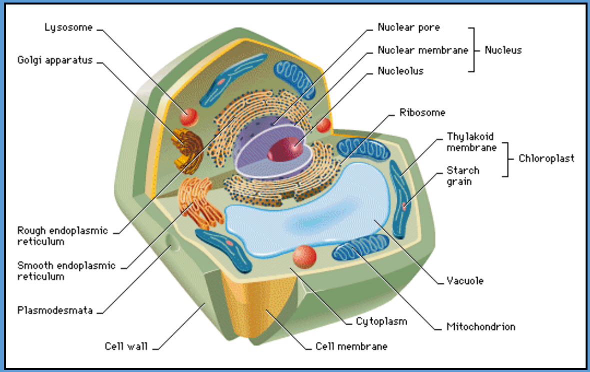
- embedded in 3D tissues
- rigid and impermeable cell wall
- various secondary metabolites



animal cell

MODIFICATION OF FCM PROTOCOLS

- tissue desintegration
- cell wall removal
- nuclei isolation

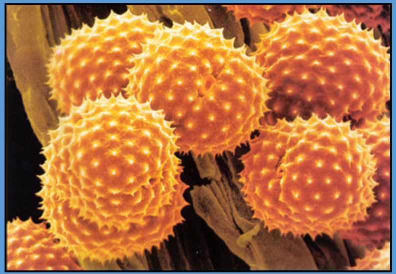


plant cell

What can be analyzed?

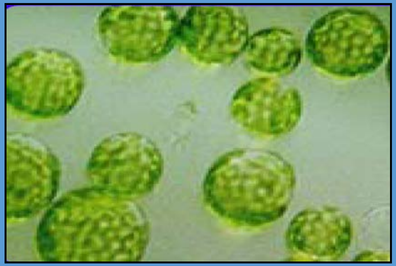
- **intact cells (pollen, sperm cells)**

  (but: large size, irregular shape, low permeability of the cell wall, autofluorescent pigments)



- **protoplasts**

  (but: laborious, not universal)



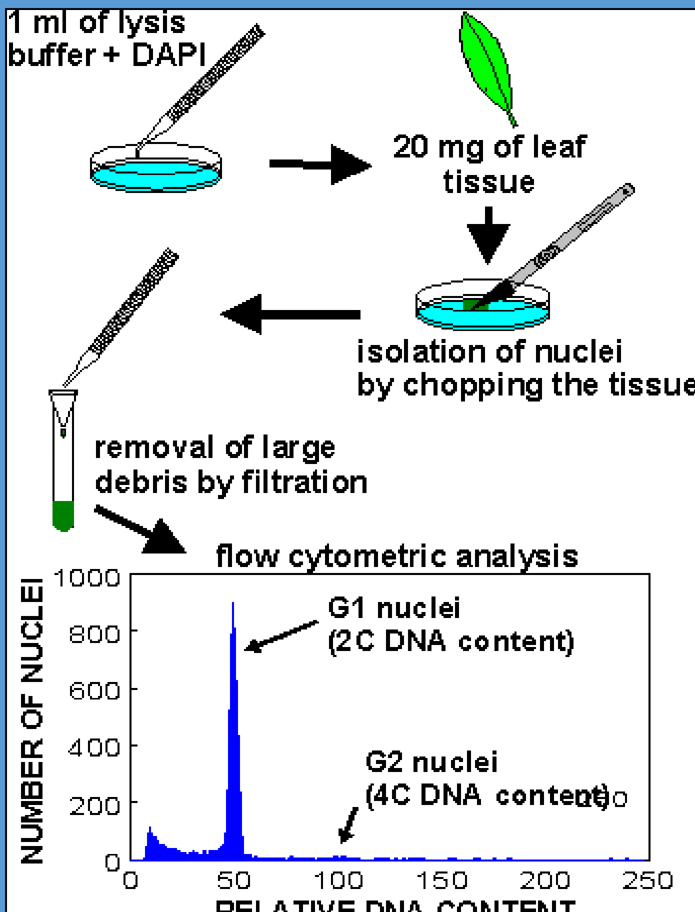
- **protoplast-derived nuclei**

- **<u>nuclei from intact tissues</u>**

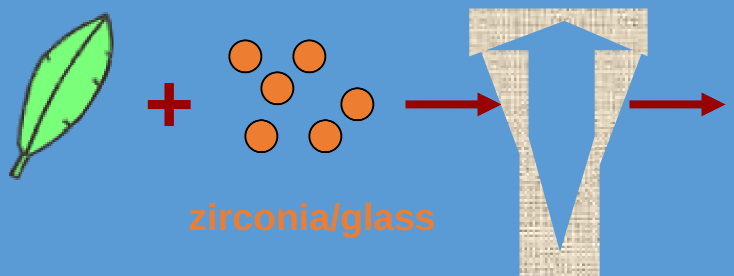
  Galbraith et al. (1983)

M e t h o d o l o g y

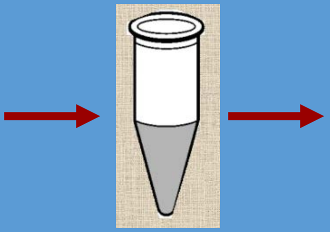
- tissue chopping



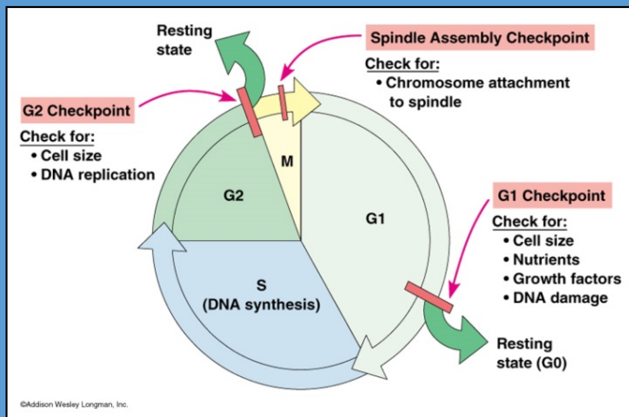
- bead-beating method

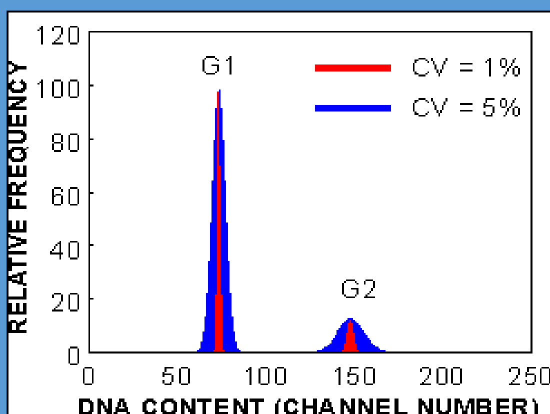


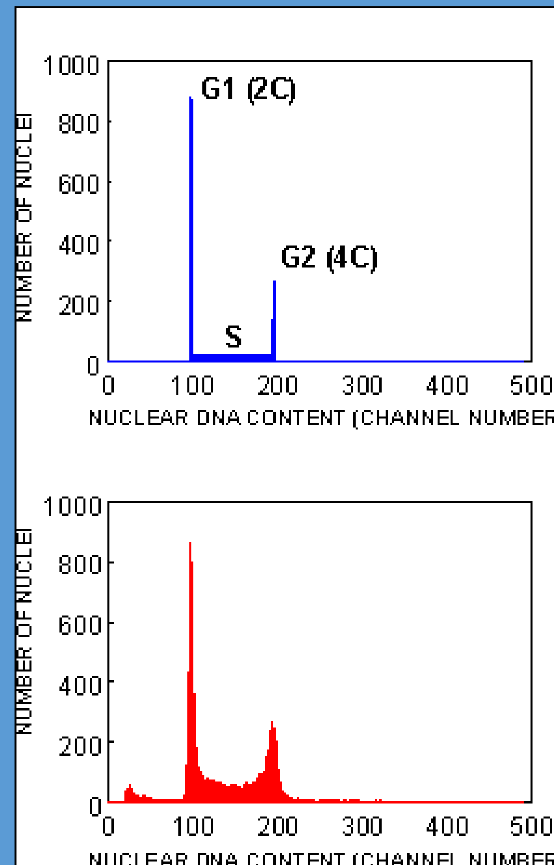
- ultrasonic disruption



DNA content distribution







Theoretical

Actual

www.ueb.cas.cz/Olomouc1

Accuracy: coefficient of variation (CV)

Resolution threshold (%): 2 x CV

Advantages / Limitations

- high speed
- statistically representative
- convenient sample preparation
- non-destructiveness
- range of tissue types
- mitotically inactive cells
- mixed samples detection
- reasonable price

- lack of visual control
- secondary metabolites
- detection of aneuploidy
- price of instruments
- (use of non-fresh material)

Contributions of FCM

- increased number of characters

  (ploidy, genome size, AT/GC content)

- increased number of individuals (population level)

↓

- improved detection and delineation of taxa

- larger spatial and temporal scales

- more robust inference of phylogenetic relationships

↓

- new research questions

Overview of applications

Determination of closely related taxa → different ploidy level
→ same ploidy level

Determination of hybrid plants → heteroploid crosses
→ homoploid crosses

Screening for rare cytotypes

Cytotype distribution pattern at various spatial scales
Dynamics of mixed-ploidy populations

Detection of aneuploid individuals
Detection of sex in dioecious plants
Detection of endopolyploidy
Detection of agmatoploidy

Breeding mode detection

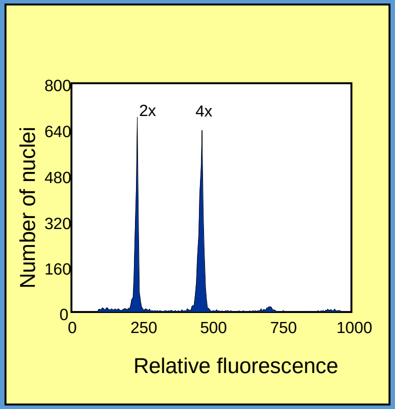
Genome composition in allopolyploid taxa
Base composition (AT / GC ratio)

C- and Cx-values determination

Heteroploid groups

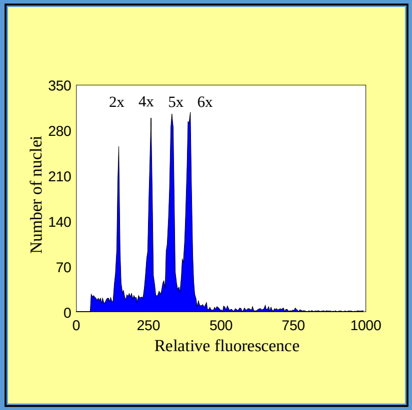
- taxonomic marker (grasses, geophytes, parasites)
- species determination and delineation

Anthoxanthum
2x: *A. alpinum*
4x: *A. odoratum*



Trávníček et al. (in prep.)

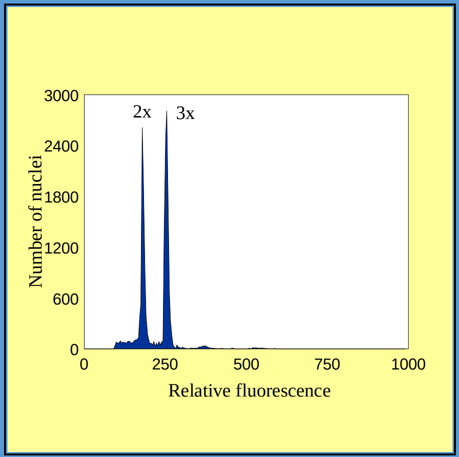
Vaccinium
2x: *V. microcarpus*
4x-6x: *V. oxycoccos*



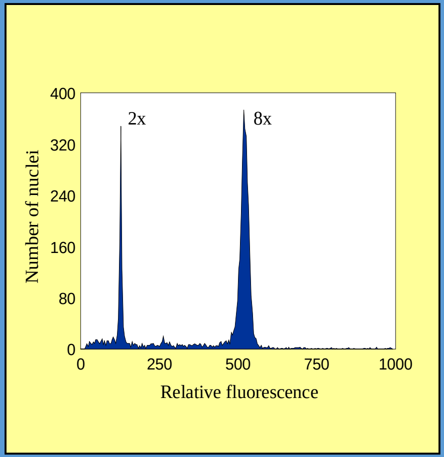
Suda & Lysák (2001)

Rare cytotypes

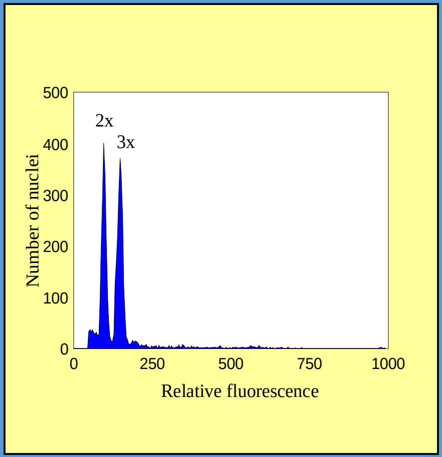
- **detection of cryptic diversity**
- **evolutionary role of minority ploidies**



Lythrum - 3x



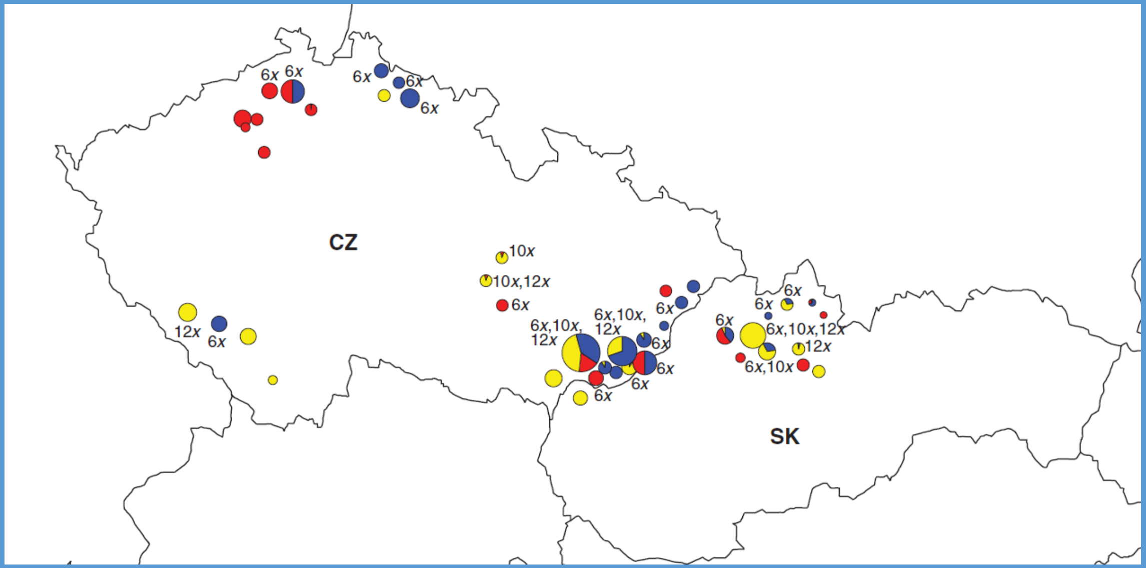
Campanula patula - 8x

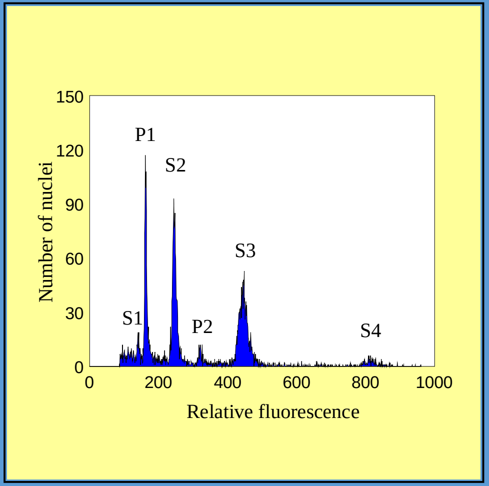


Vaccinium - 3x

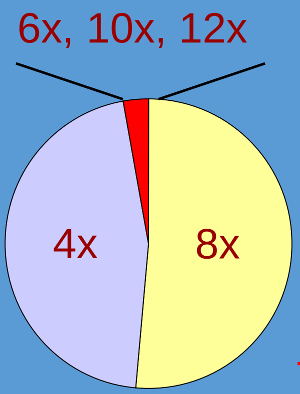
Rare cytotypes

- ➢ **use in plant conservation**





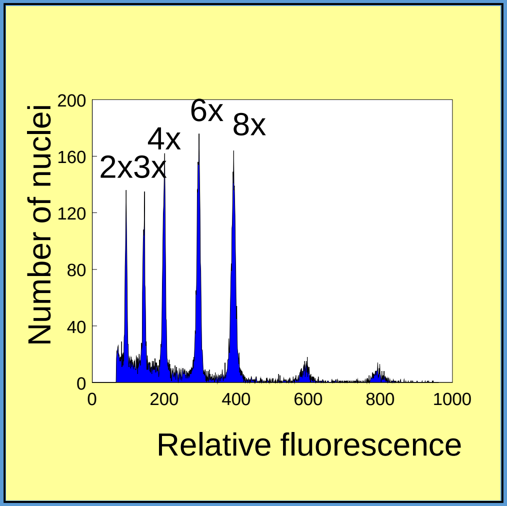
Gymnadenia conopsea



Trávníček et al. (2011)

Ploidy variation

➢ **knowledge of overall ploidy variation**



Rauchová et al.

Cape *Oxalis*

➢ **80 species: 33 multiple ploidy levels (2x - 20x)**

➢ **up to 7 different cytotypes within a species**

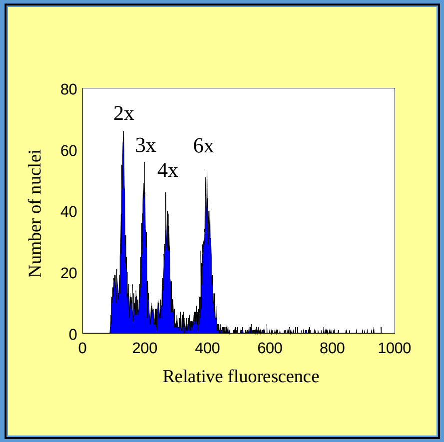
➡ **new insights into evolutionary drivers**

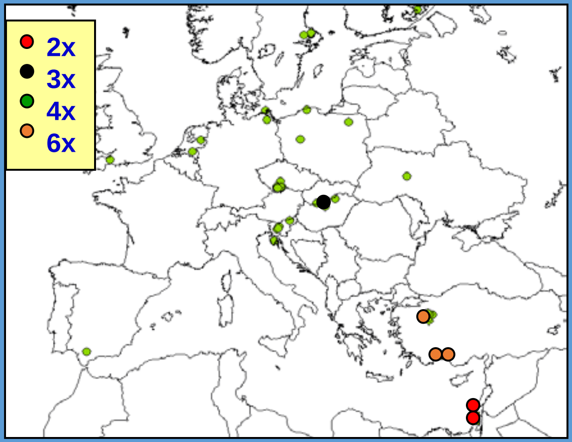
Invasion biology

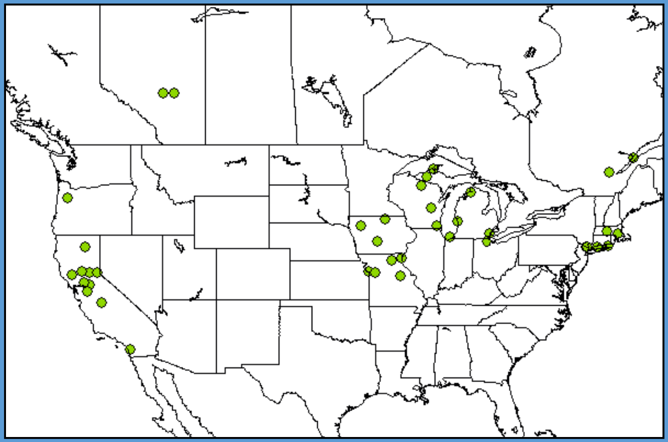
- ➢ **determinants of invasiveness**
- ➢ **cytotype composition in primary vs. secondary areas**

Lythrum salicaria

- ➢ Europe + Asia (native): 2x, 3x, 4x, 6x
- ➢ North America (invasive): 4x



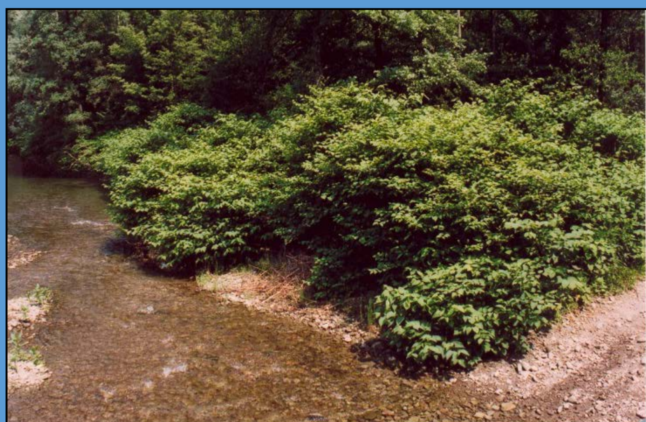


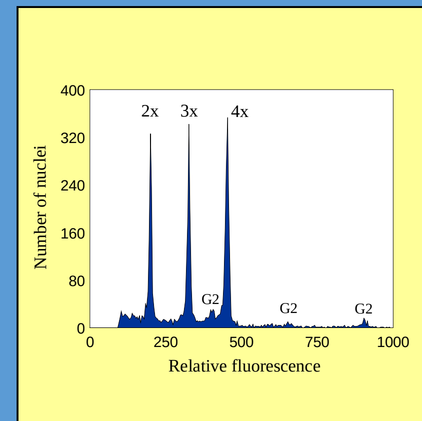


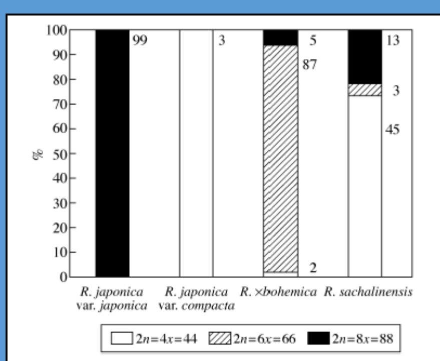
Kubátová et al. (2008)

Invasion biology

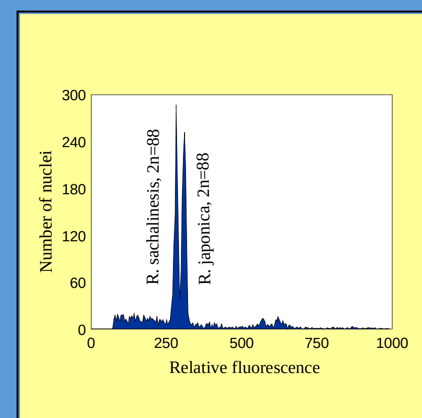
- evolution in secondary areas
- ploidy-specific behaviour







Reynoutria

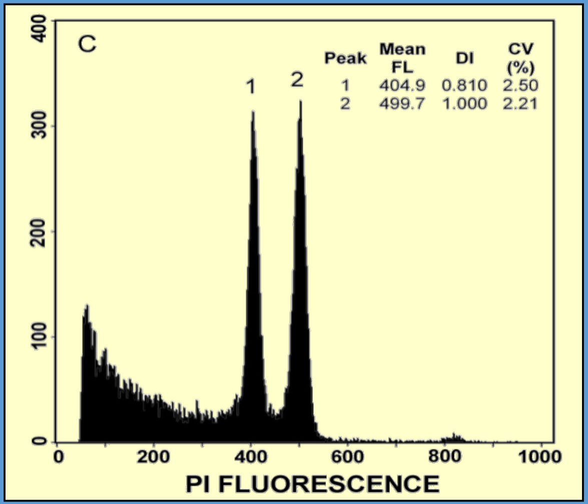


Mandák et al. (2003)

Invasion biology

Oxalis pes-caprae

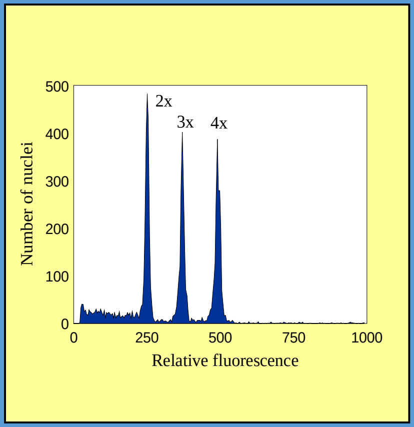
- South Africa (native): 4x > 2x > 5x
- Europe (invasive): 5x > 4x



Heteroploid hybridization

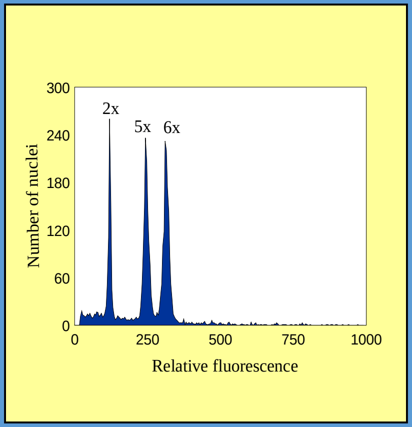
➤ **reliable detection of interspecific hybrids**

Lamium **- 3x**



Rosenbaumová et al. (2004)

Vaccinium **- 5x**



Suda & Lysák (2001)

Ploidy dynamics

Experimental hybridization:

6x (facultative apomict)

x **4x** (obligate apomict)

Hieracium **subg.** ***Pilosella***

Offspring ploidy:

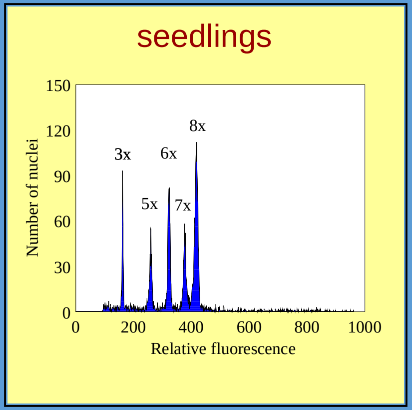
6x (apomixis)

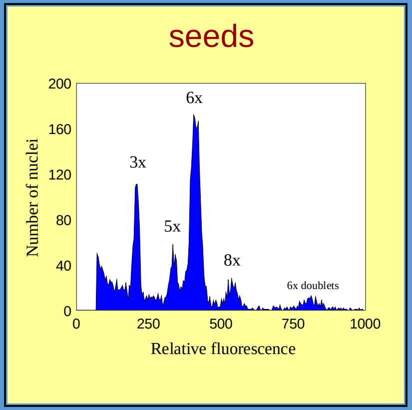
3x (haploid parthenogenesis)

5x (reduced gametes)

7x (reduced + unreduced gametes)

8x (unreduced + reduced gametes)

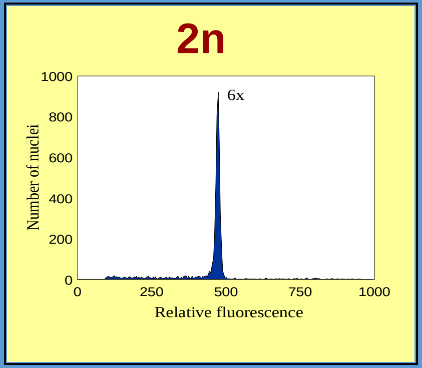




Krahulcová et al.

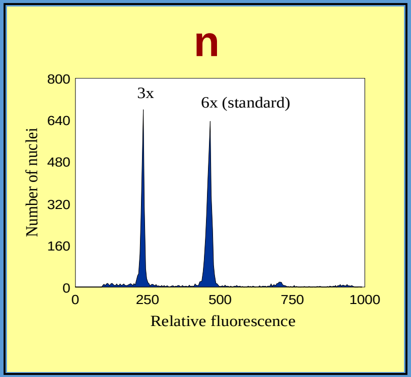
Ploidy dynamics

Hieracium* subg. *Pilosella



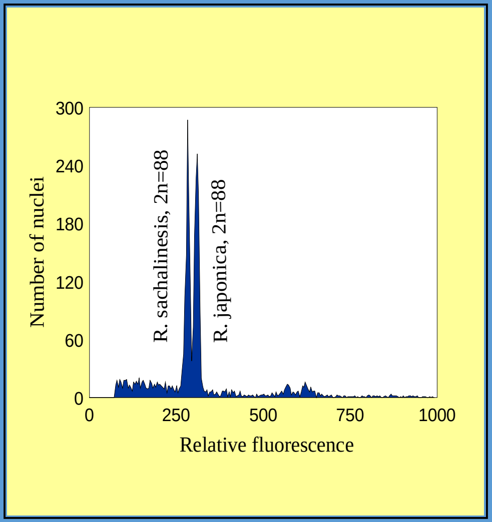
Polyploidization

Haploid parthenogenesis

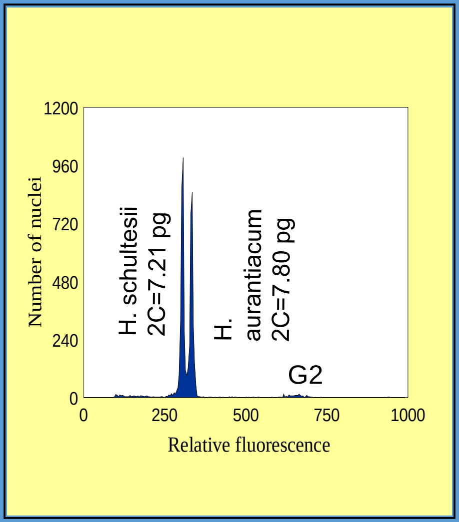


Homoploid groups

- discrimination between species with the same number of chromosomes
- determination of plant fragments, roots, juvenile plants, etc.



Reynoutria



Hieracium* subg. *Pilosella

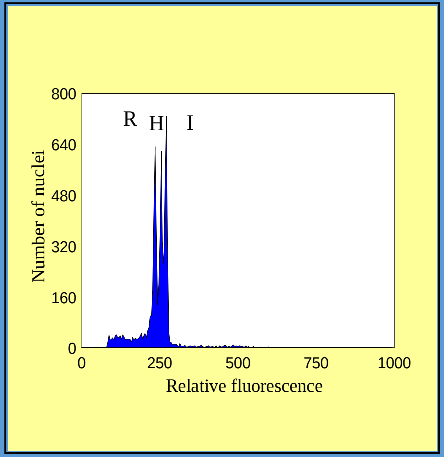
Suda et al. (2010)

Suda et al. (2007)

Homoploid hybridization

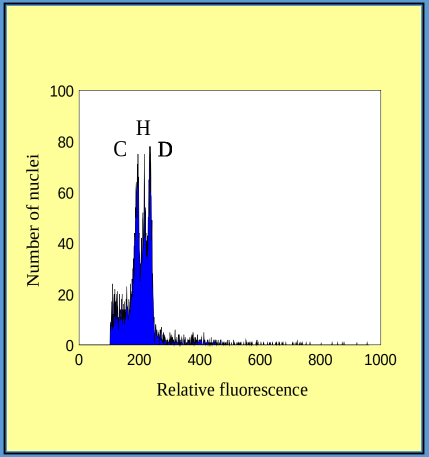
- discrimination between homoploid hybrids and parental species (threshold ~ 4%, parental difference ~ 7%)

Elytrigia repens x *E. intermedia*



Mahelka et al. (2005)

Dryopteris carthusiana x *D. dilatata*

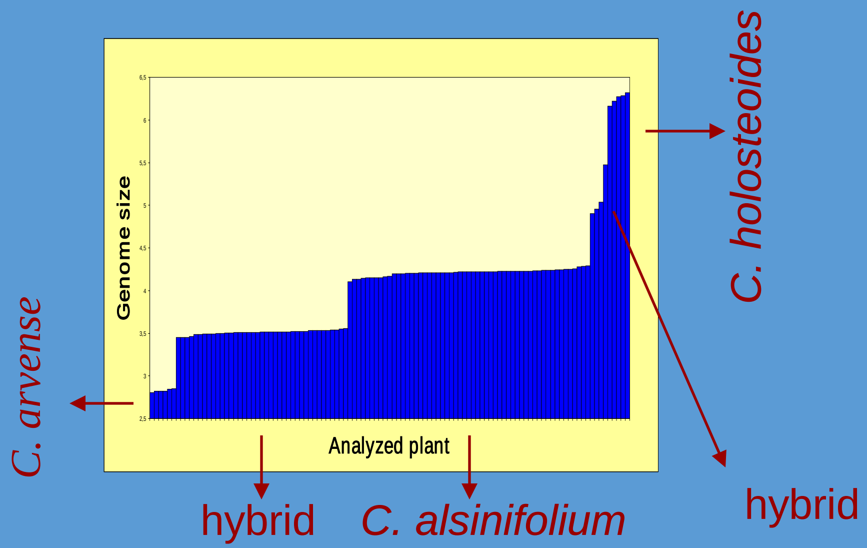


Ekrt et al. (2010)

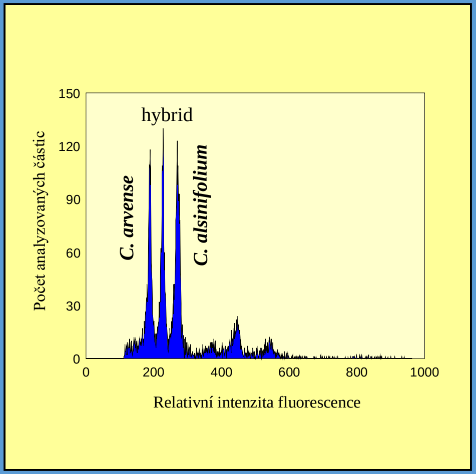
Homoploid hybridization

- **conservation implications**

  (risk of interspecific hybridization)



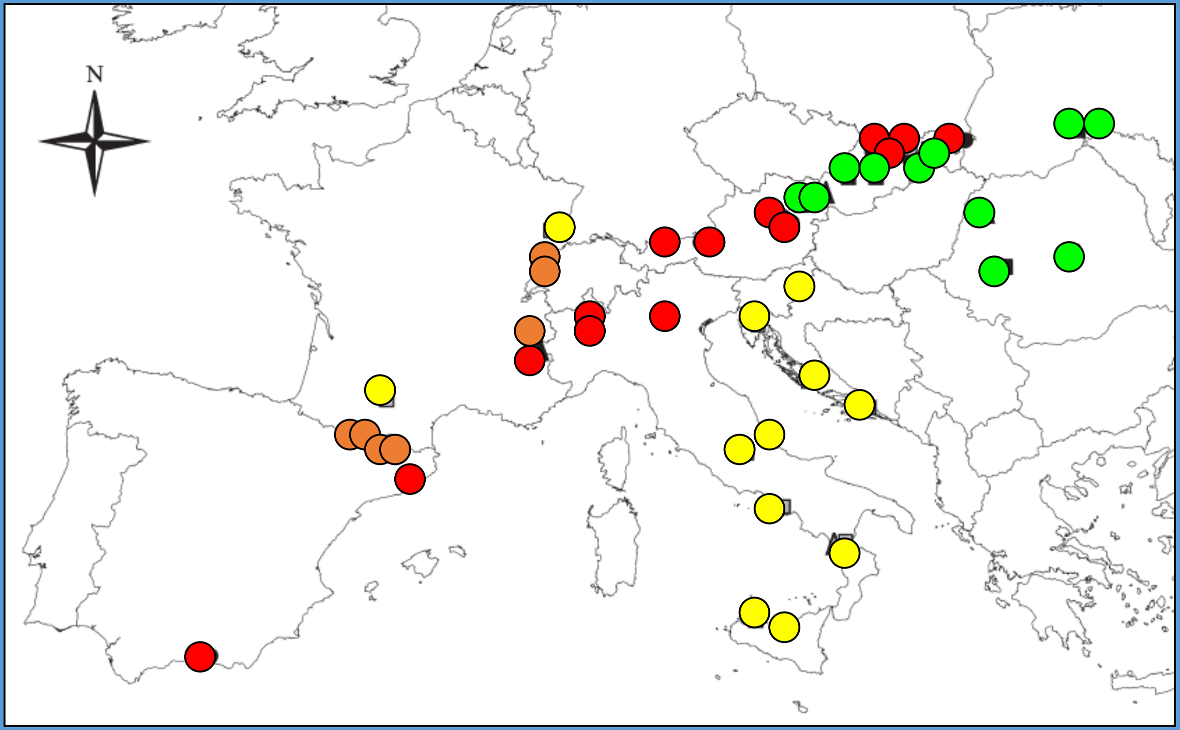
***C. alsinifolium* x *C. arvense* (2n =72)**



Vít et al. (2014)

Cryptic diversity

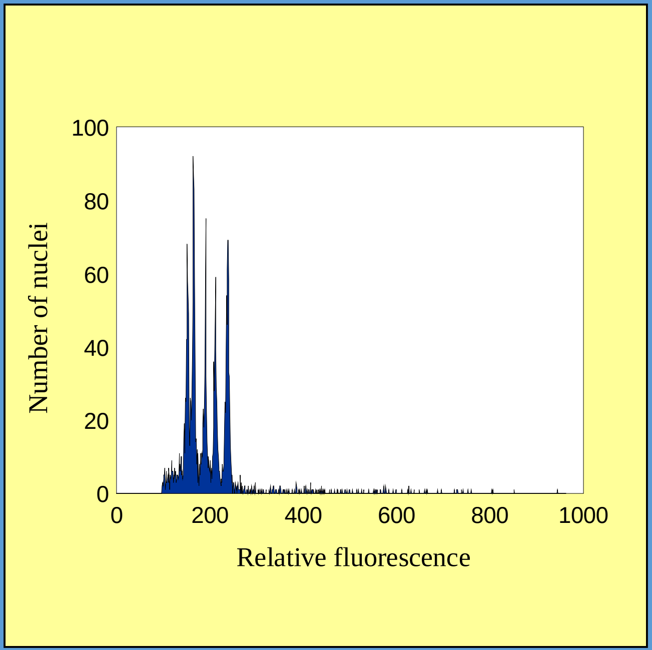
Picris hieracioides



Slovák et al. (2008)

High-altitude	Low-altitude
large GS	large GS
small GS	small GS

Cryptic diversity



Ellis et al. (in prep.)

Gorteria diffusa

Origin of polyploids

➢ **putative parents in allopolyploids**

A. puberula

2C = 1.18 pg

A. adfinis

2C = 1.15 pg

Androsace brigantiaca

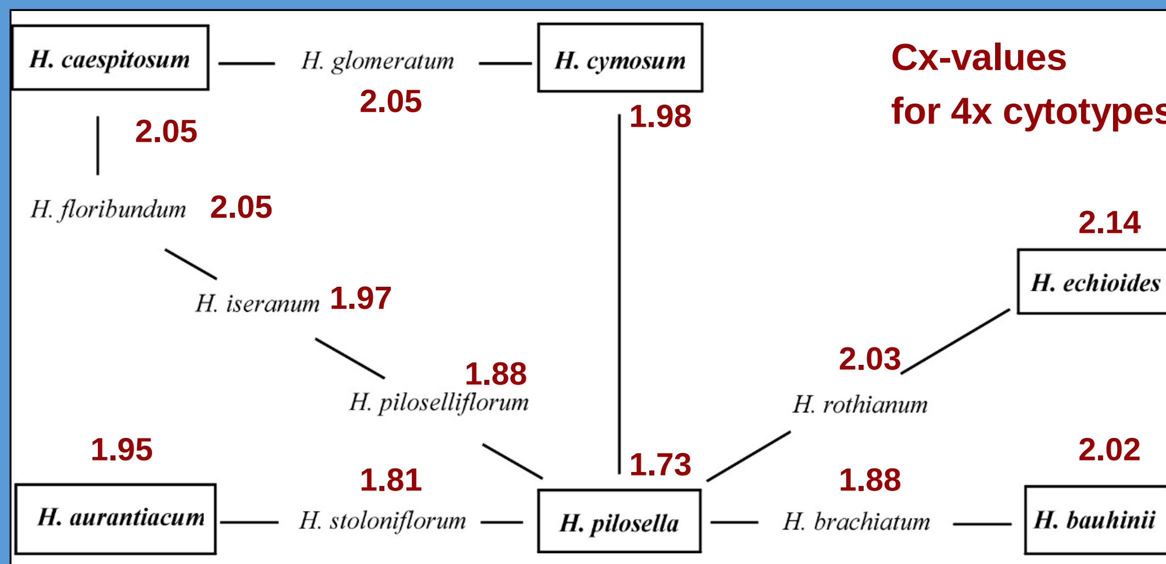
2C = 2.33 pg

Dixon et al. (2008)

Evolutionary relationships

Basic species

Intermediate (hybridogenous) species



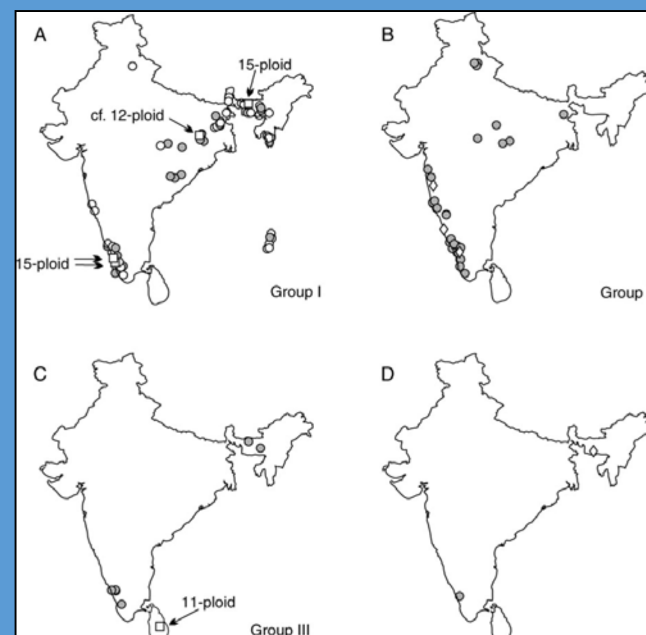
Hieracium* subg. *Pilosella

Suda et al. (2007)

Evolutionary relationships

Leong-Škorničková et al. (2007)

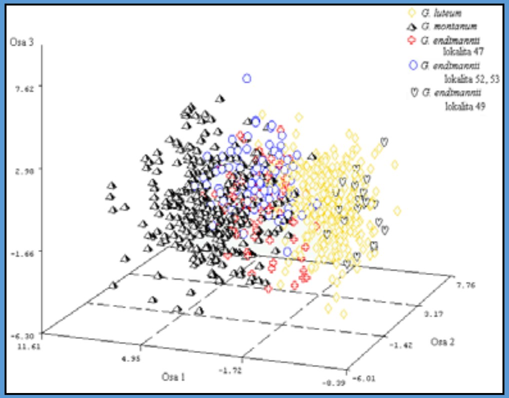
- 6 different ploidy levels
- 3 genome size (Cx-values) groups (correlation with geography)

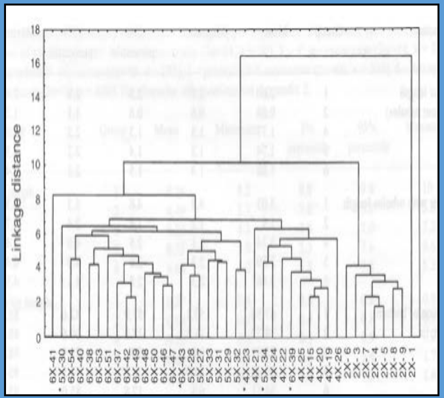


Links to other techniques

Multivariate morphometrics

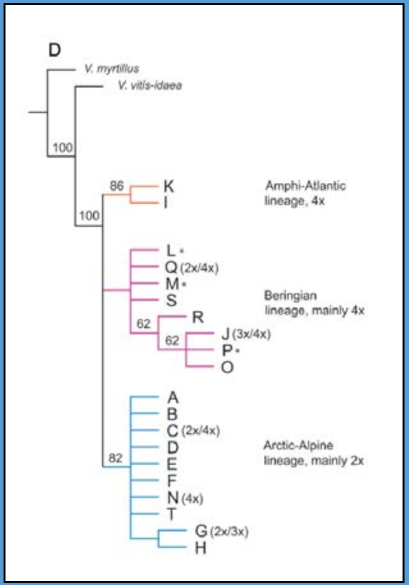
- insights into phenotypic variation
- species- and cytotype-specific characters
- determination of cryptic taxa





Molecular methods

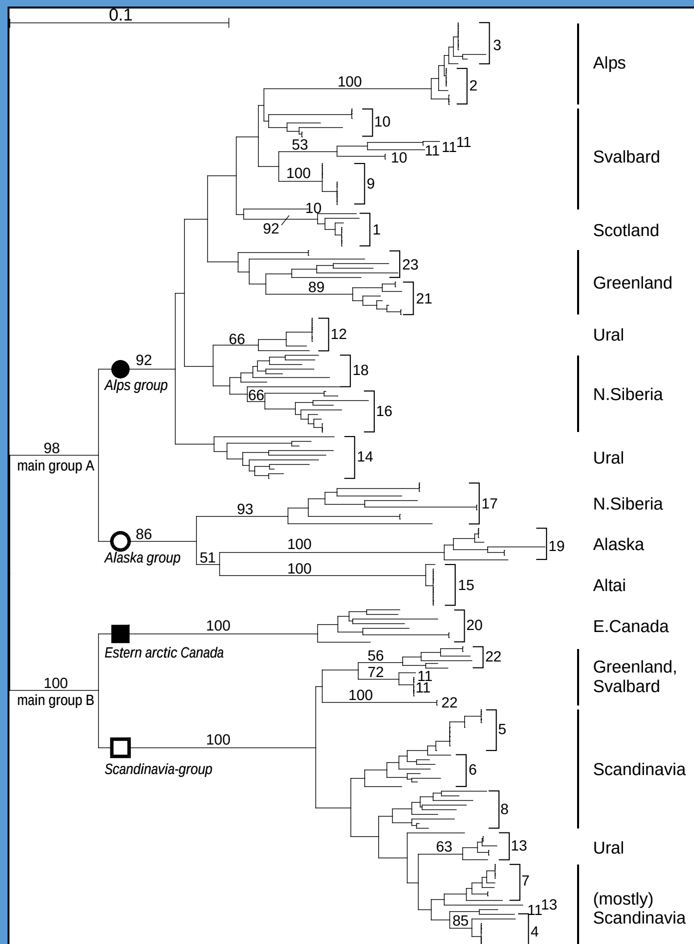
- genome size evolution
- ploidy dynamics



Resolving phylogeny

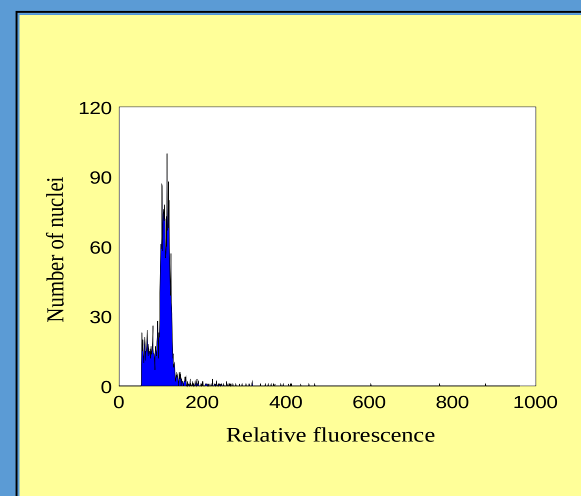
2C = 1.48 pg

2C = 1.39 pg



Juncus biglumis

Schönswetter et al. (2007)

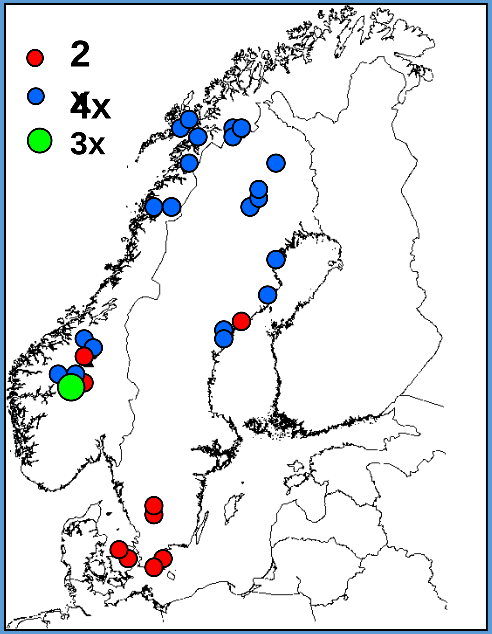


Cytotype distribution

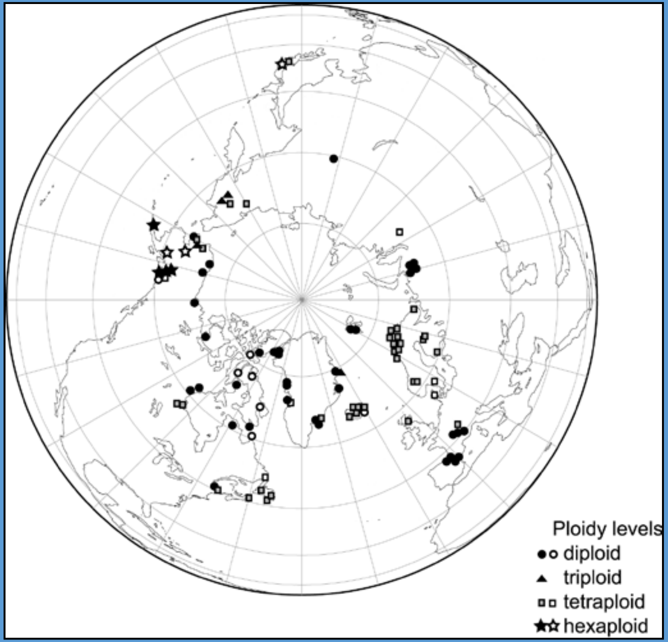
- variation at large spatial scales

Vaccinium uliginosum

Empetrum



Suda (2002)

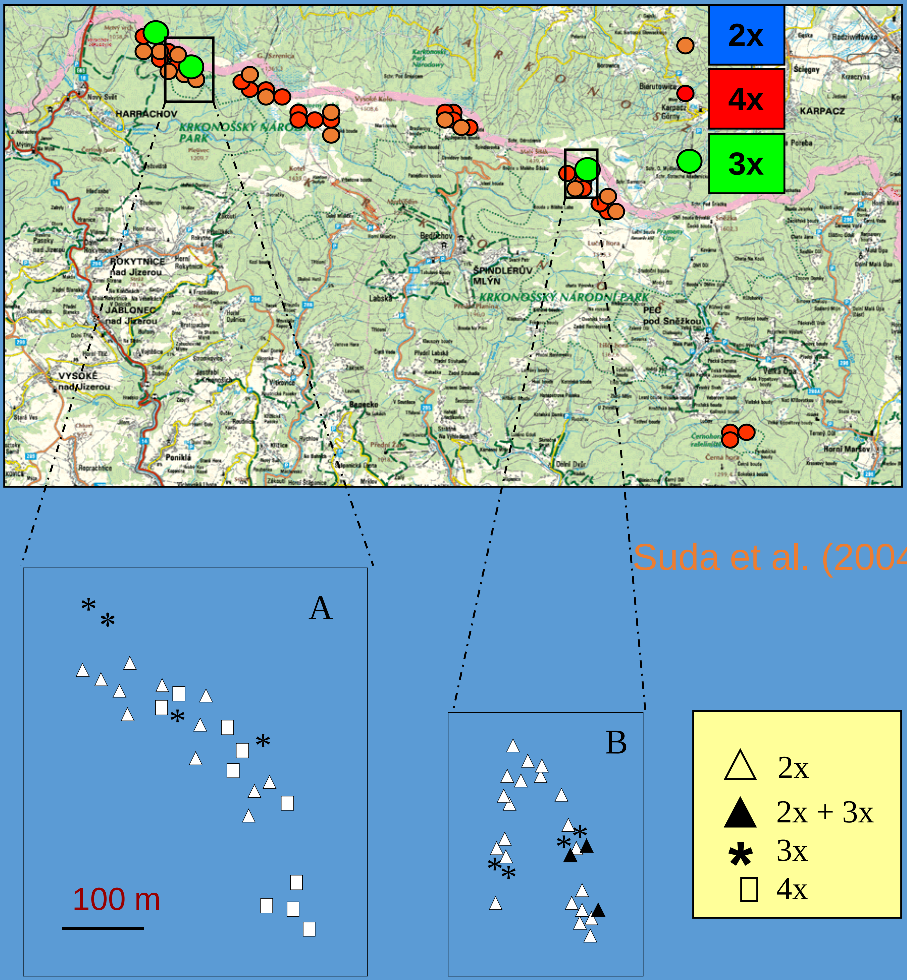


Eidesen et al. (2007)

Cytotype distribution

- variation at fine spatial scales

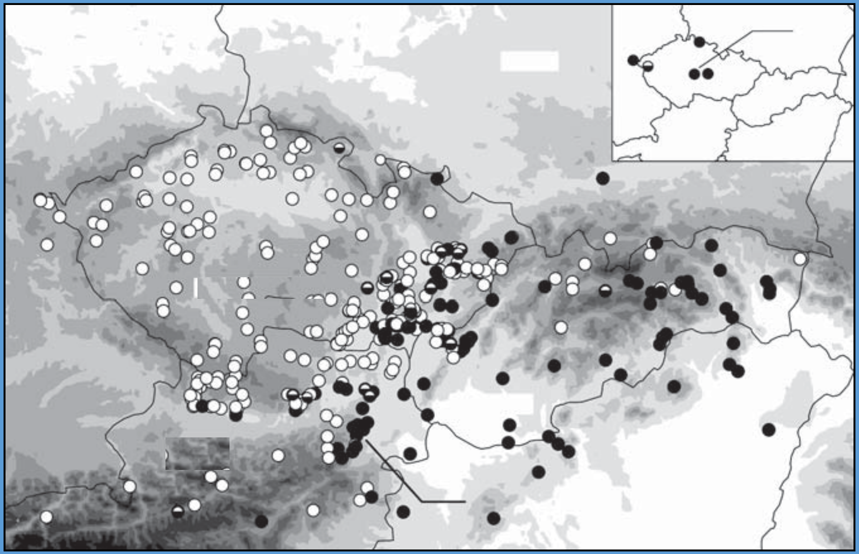
Empetrum

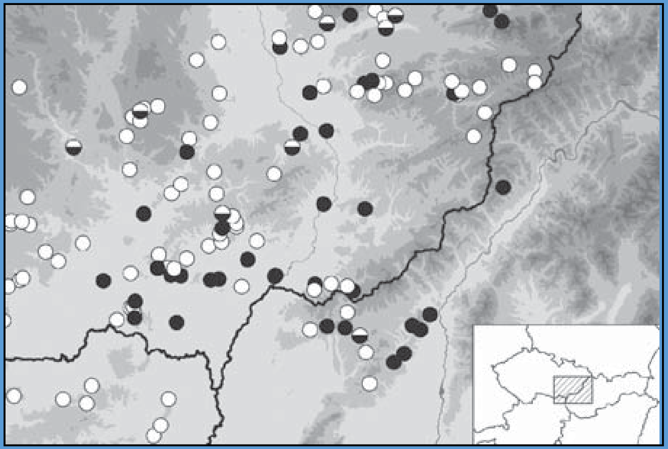


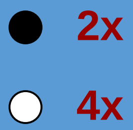
Suda et al. (2004)

Sympatric occurrence

Knautia arvensis







Kolář et al. (2009)

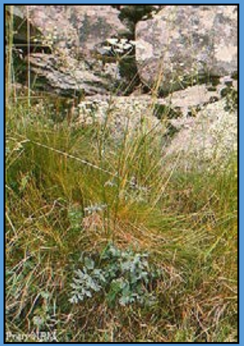
Sympatric occurrence

Objectives

- life history dynamics and demographic parameters
- mechanisms of reproductive isolation
- inter- and intra- cytotype interactions
- interactions with other trophic levels
- ecological and functional aspects of polyploidy

Sympatric occurrence

Campanula patula
2x + 4x



Pimpinella saxifraga
2x + 4x

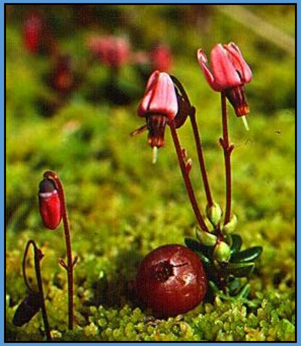
Anthoxanthum
2x + 4x

Vicia cracca
2x + 4x

Common phenomenon of high evolutionary significance

Galeobdolon
2x + 3x + 4x

Hieracium echioides
2x + 3x + 4x + 5x

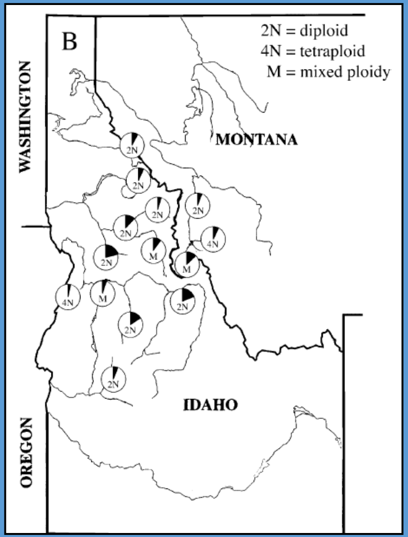


Oxycoccus
2x + 4x + 5x + 6x

Gymnadenia
4x + 6x + 8x + 10x +12x

Trophic interactions

- ploidy-specific infestation
- different pollinator suites



Heuchera grossulariifolia

Pollinator	Diploid mean	Tetraploid mean
Hymenoptera		
Andrena buckelli	0.90	0.80
A. nigrocaerulea	0.60	0.40
Bombus spp.	0.40	0.80
Bombus bifarious nearticus	0.25	1.25[a]
B. centralis queens	0.44	1.38[a]
B. centralis workers	1.20	0.10[a]
Dolichogenidea spp.	1.50	1.50
Lasioglossum spp.	1.68	0.71[a]
Nomada spp.	1.39	0.62
Lepidoptera		
Greya politella	0.25	1.25[a]
Diptera		
Bombyllius major	0.27	1.73[a]

Greya politella

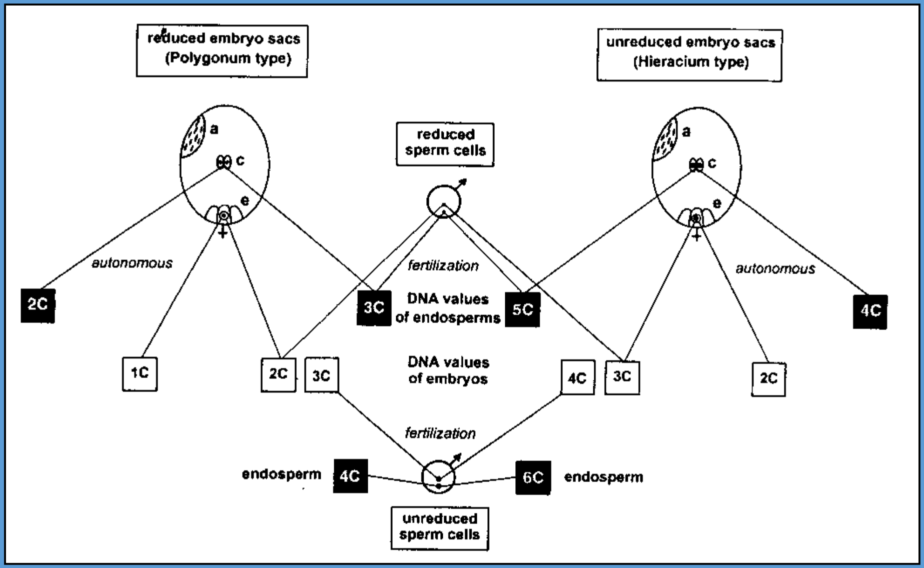
Greya piperella

Eupithecia misturata

Thompson et al. (1997, 2004)

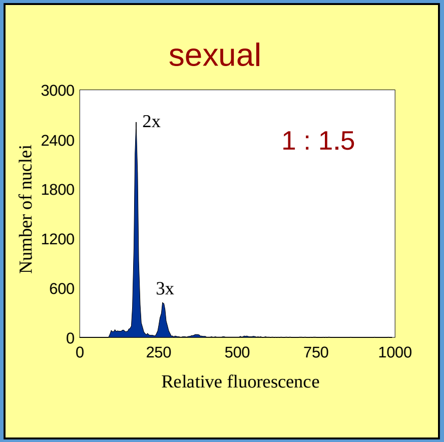
Reproductive pathways

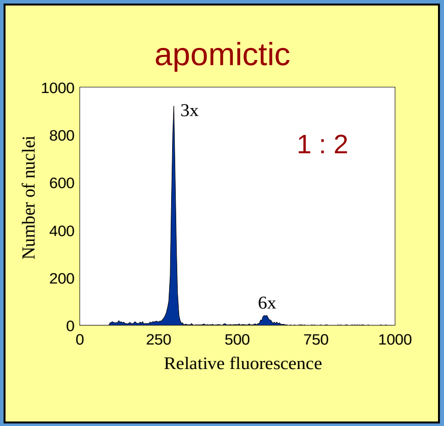
- mature seeds ➡ ploidy ratio embryo / endosperm
- sexual vs. apomictic individuals
- reduced vs. unreduced gametes



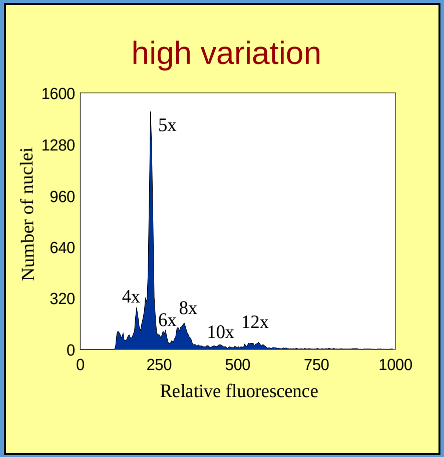
Matzk et al. (2000)

Reproductive pathways





Taraxacum

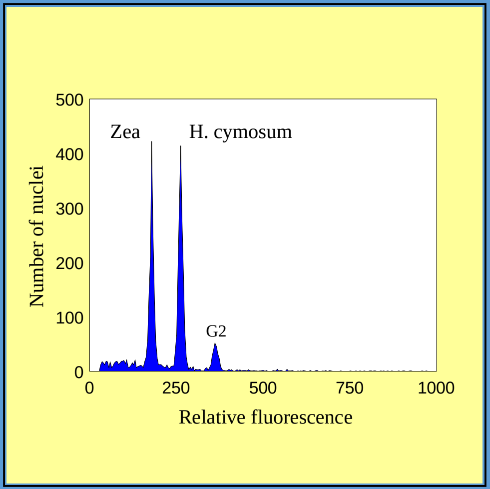


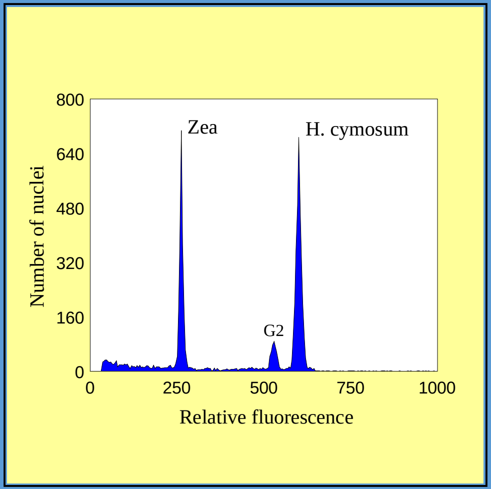
Hypericum

Base composition

- variation 52.8 - 69.9 % AT
- useful marker at higher taxonomic ranks (families)
- usually similarities among species

Propidium iodide





DAPI

Sex determination

Arecaceae

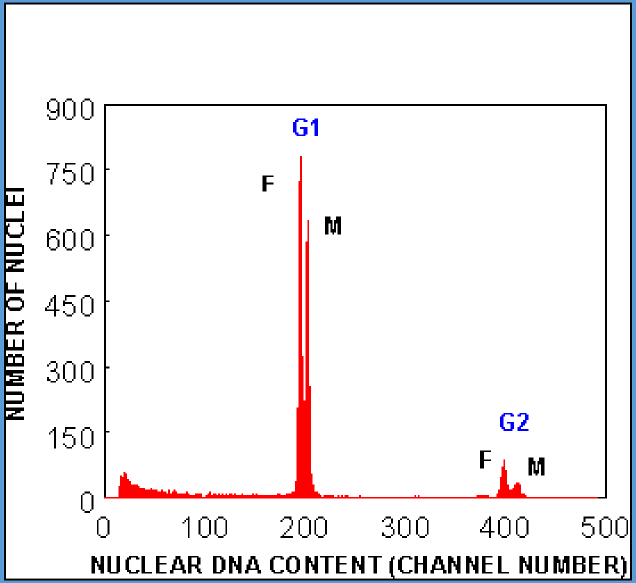
Cannabaceae

Caryophyllaceae

Cucurbitaceae

Loranthaceae

Polygonaceae



www.ueb.cas.cz/Olomouc1

***Silene latifolia* 2n = 24**

F – XX, M – XY

A g m a t o p l o i d y

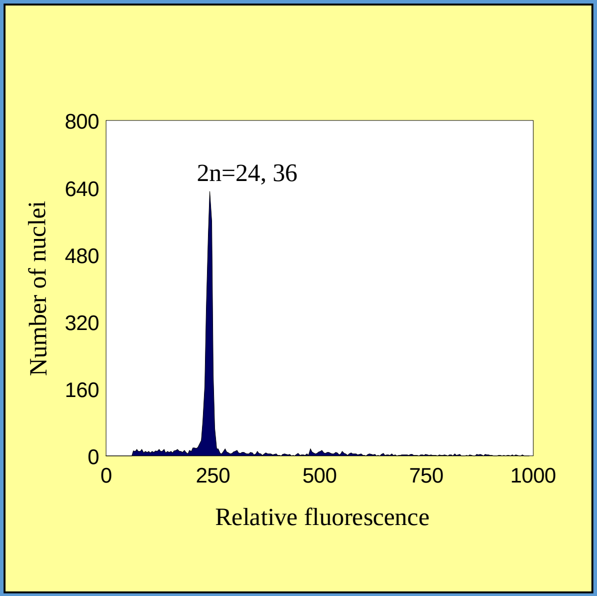
Chromosome fragmentation

- different chromosome number
- the same DNA content

Juncaceae

Cyperaceae

Juncus



How to store the samples?

SHORT-TERM

- **bagging plant tissue in moistened paper**

LONG-TERM

- **chemical preservation**

  70 / 95% ethanol

  ethanol : acetic acid

  formaldehyde

  30% glycerol

- **physical preservation**

  freezing

  desiccation

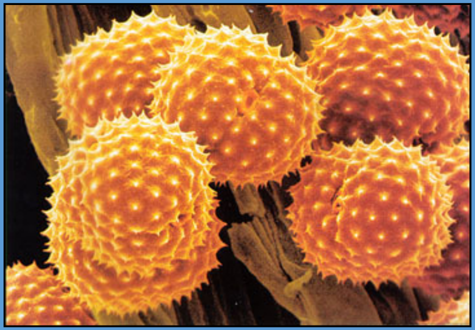
  

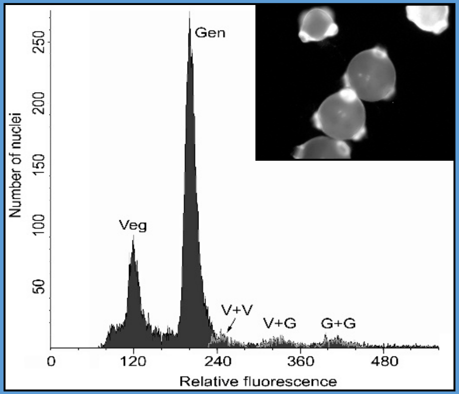
  silica-dry material

  herbarium vouchers

Other directions

- ploidy estimation in gametic tissues (pollen)
- polyploid adaptive evolution
- study of different evolutionary lineages
- dynamics of mixed-ploidy populations
- evolutionary ecology of interactions
- methodology (long-term tissue preservation)





Courtesy of P. Kron

Cardamine flexuosa and related taxa

C. flexuosa

C. occulta

EUROPE

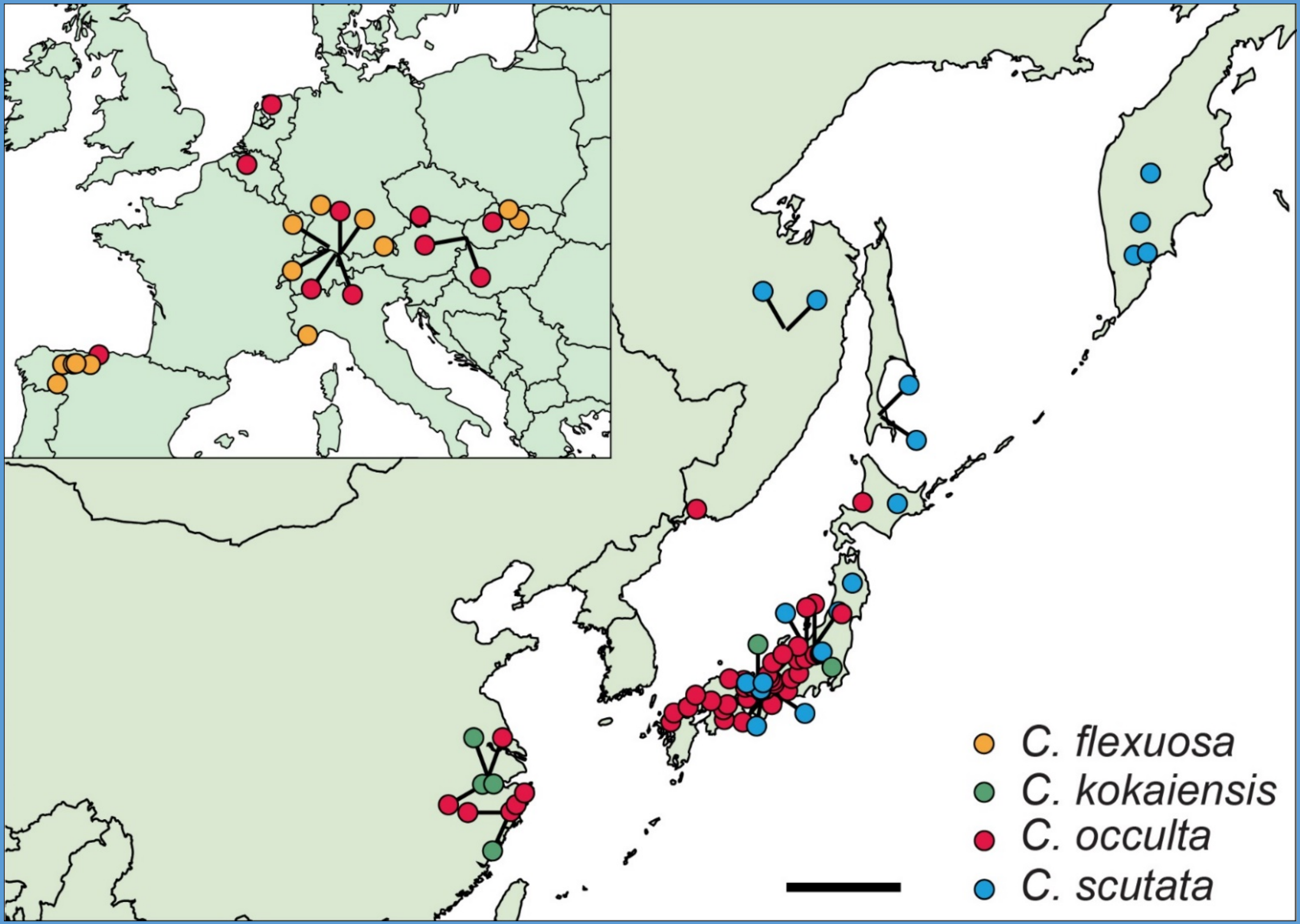
ASIA

C. scutata

C. kokaiensis

C. occulta

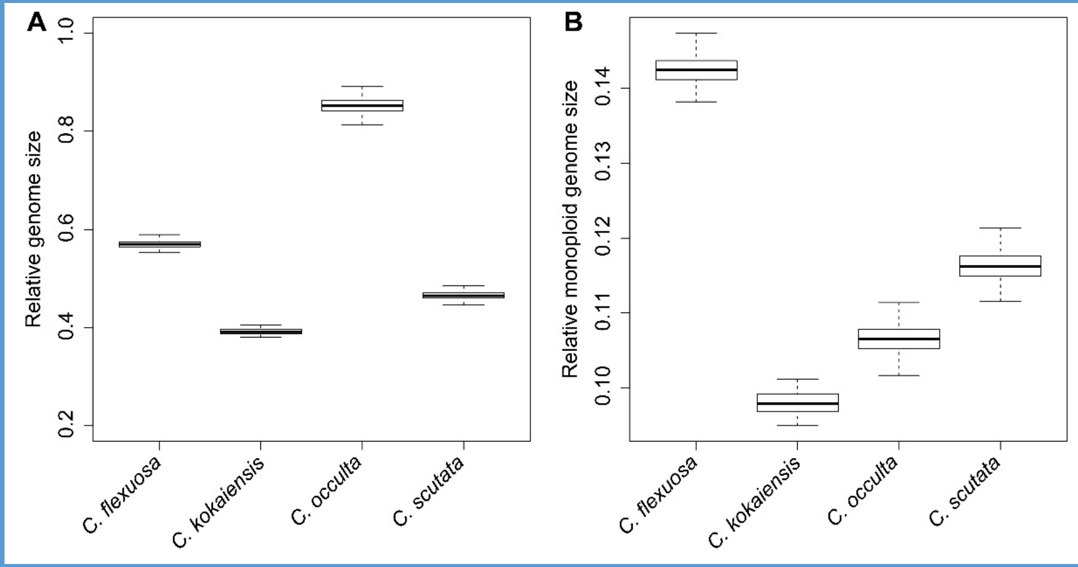
Šlenker et al., 2018, Botanical Journal of the Linnean Society, 187: 456–482.



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.

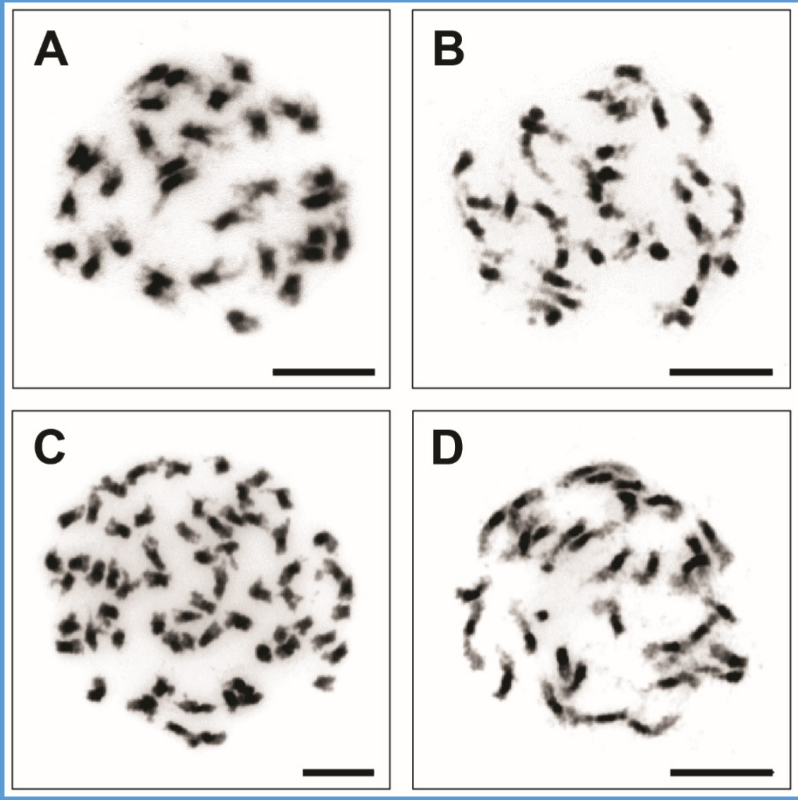
Šlenker et al., 2018, Botanical Journal of the Linnean Society, 187: 456–482.

Flow cytometry and chromosome number counts



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

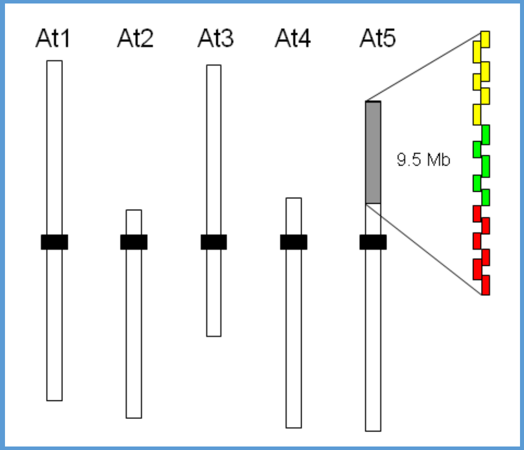
Chromosomes of (**A**) *Cardamine* ***flexuosa*** (2*n* = 32), (**B**) *C.* ***kokaiensis*** (2*n* = 32), (**C**) *C.* ***occulta*** (2*n* = 64) and (**D**) *C.* ***scutata*** (2*n* = 32) counterstained by DAPI. Scale bars indicate 10 µm.

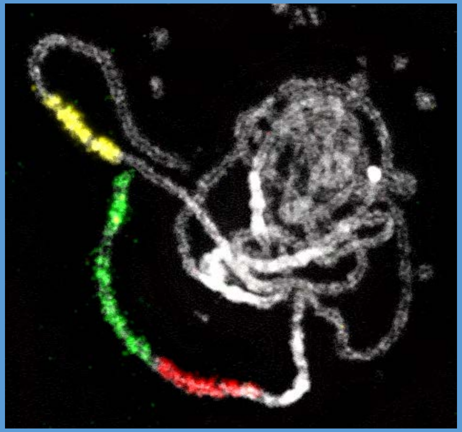


Šlenker et al., 2018, Botanical Journal of the Linnean Society, 187: 456–482.

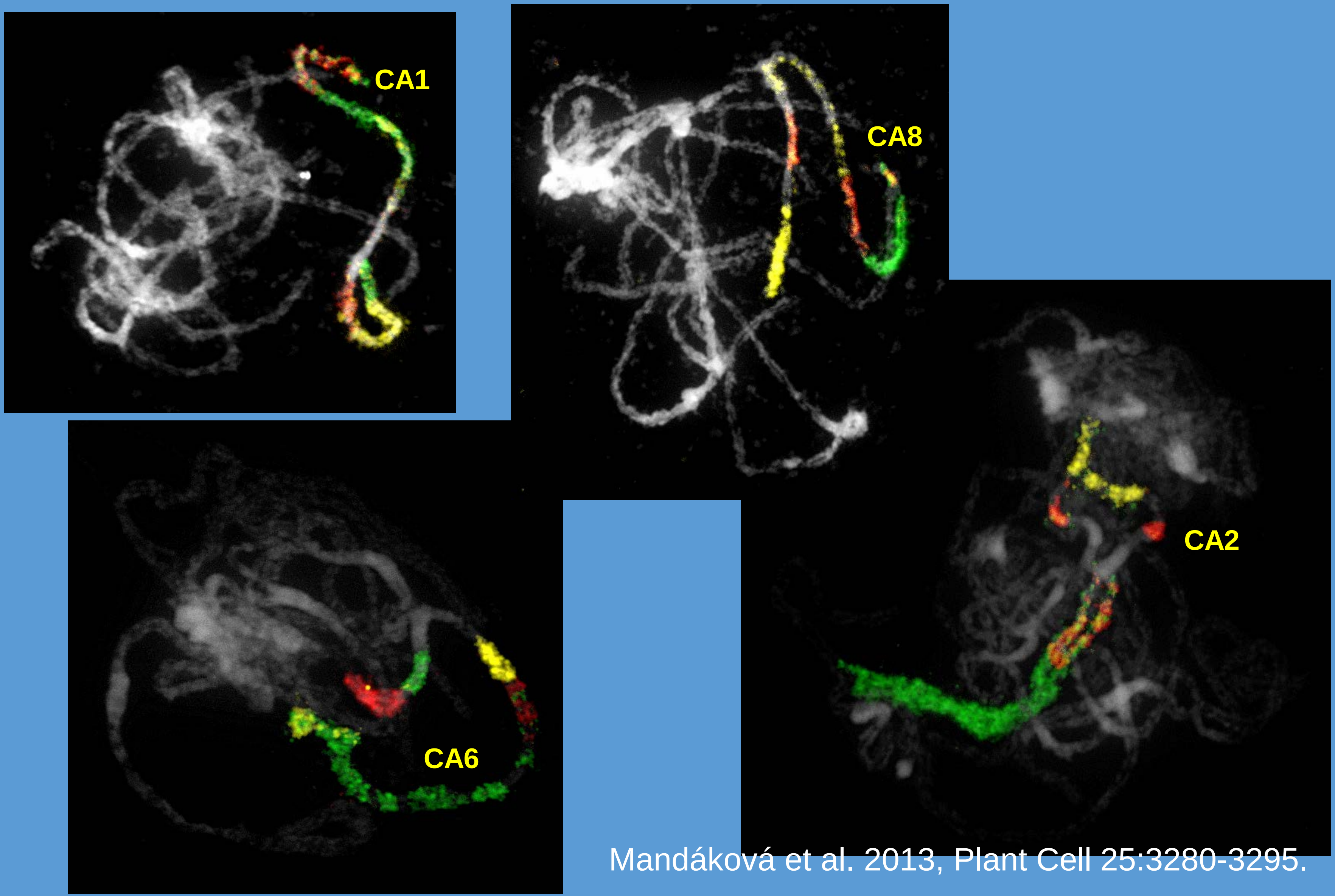
Comparative chromosome painting

Arabidopsis thaliana BACs → BAC contigs → multicolor fluorescence *in situ* hybridization on meiotic (pachytene) chromosomes





Examples of CCP in *Cardamine amara*



Mandáková et al. 2013, Plant Cell 25:3280-3295.

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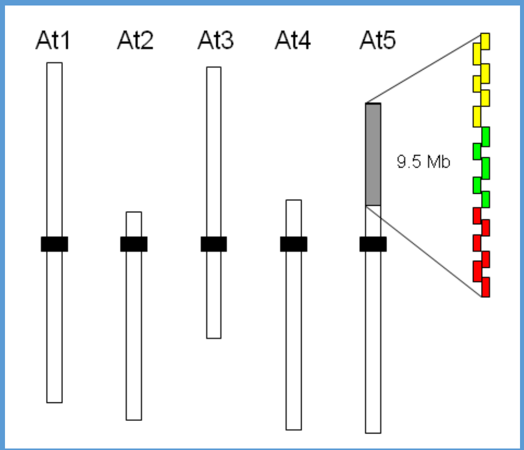
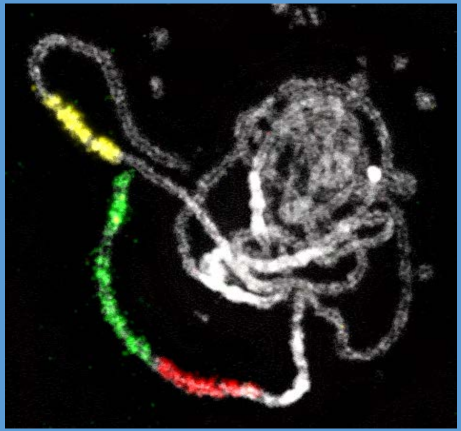
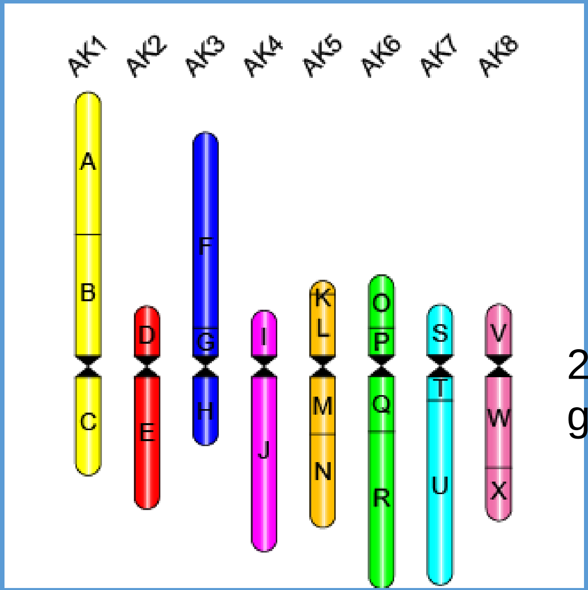
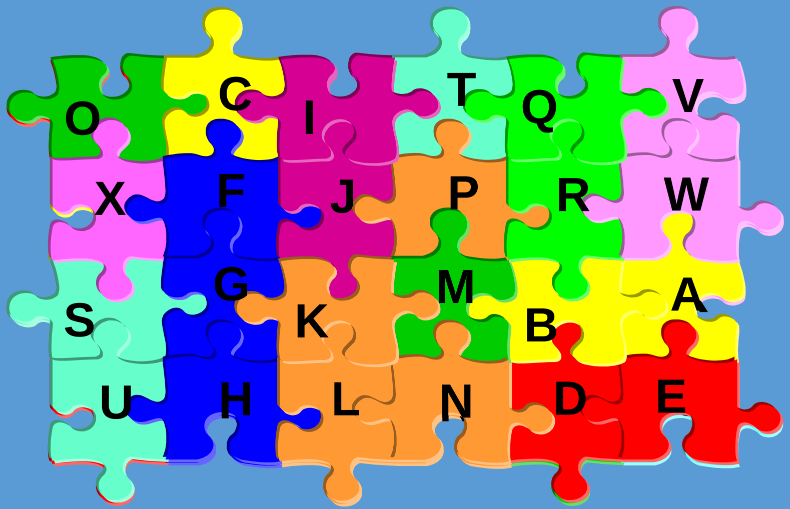




Ancestral Crucifer Karyotype (ACK, n=8)

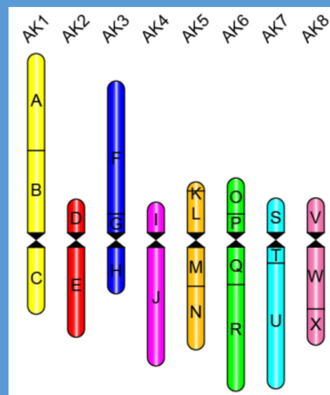


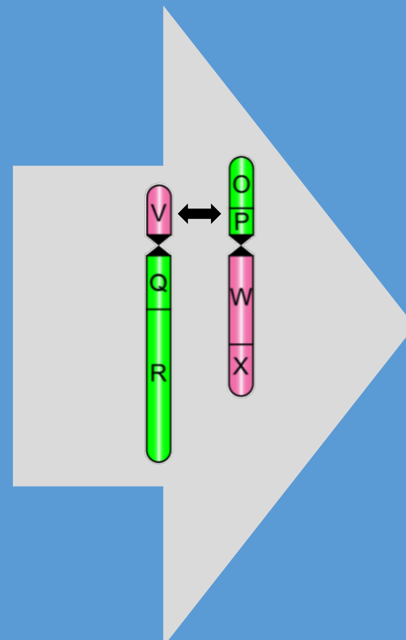
24 conserved genomic blocks



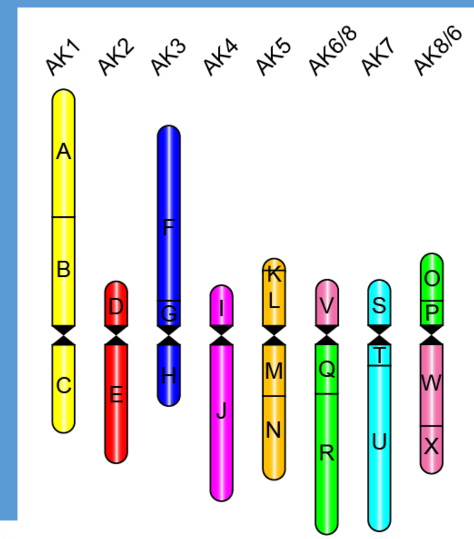
A tribal-specific reciprocal translocation in the Cardamineae

Ancestral Crucifer Karyotype (n = 8)





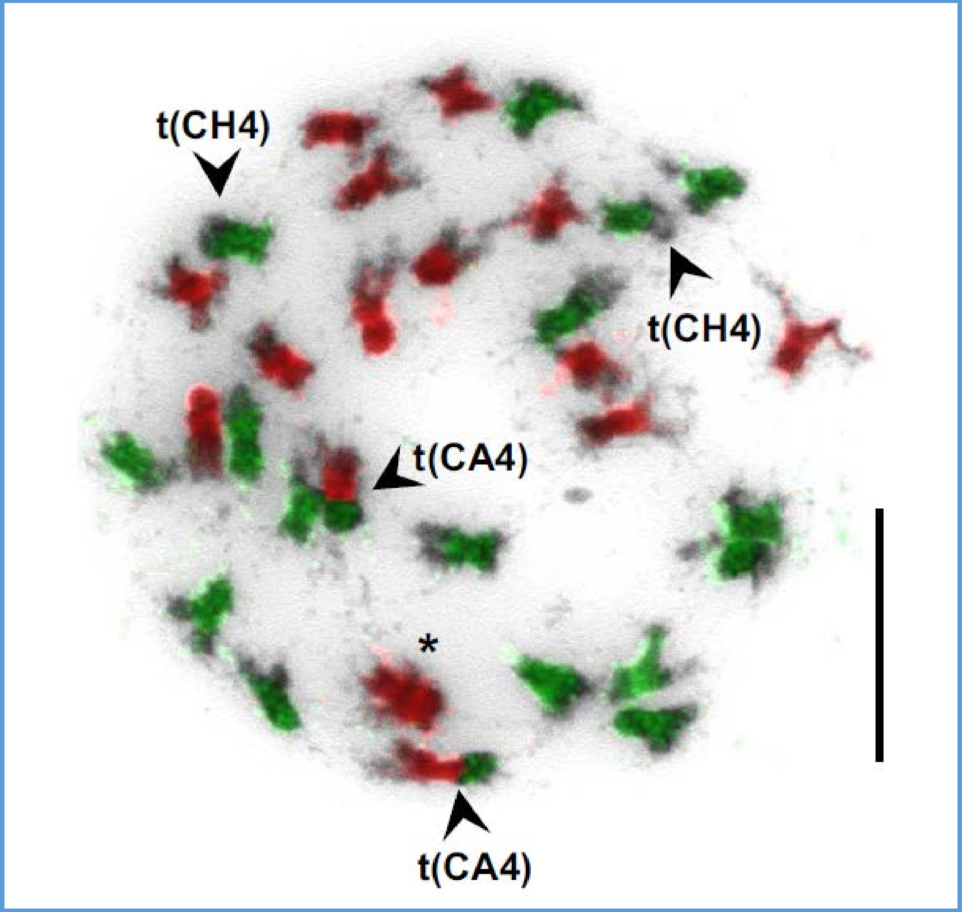
Ancestral karyotype of Cardamineae (n = 8)



Cardamineae:
12 genera (337 spp.) worldwide

Mandáková & Lysak, in prep.

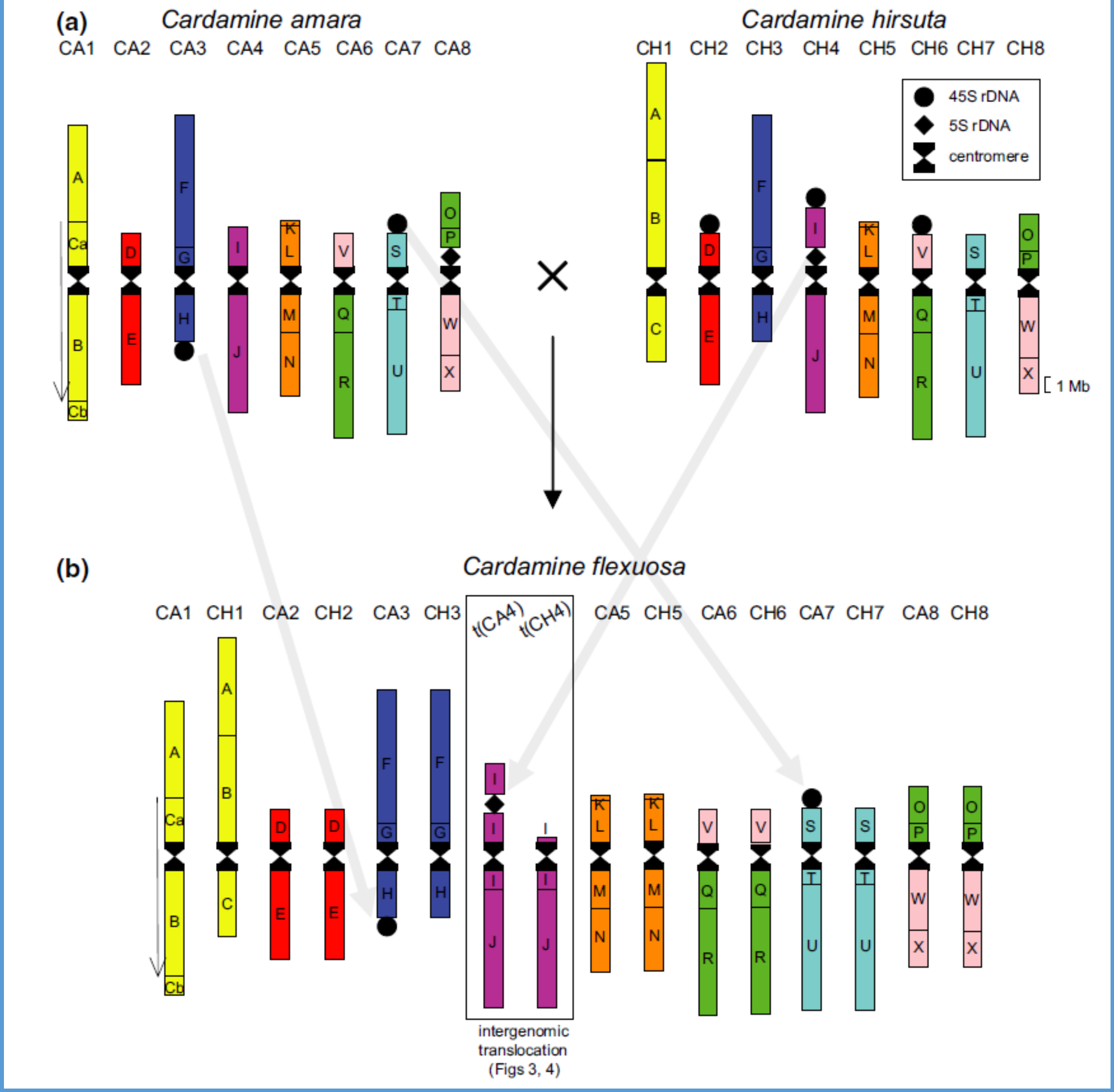
Origin of European *Cardamine flexuosa*



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.

Mandáková, Marhold & Lysák, 2014, New Phytologist 201: 982–992.

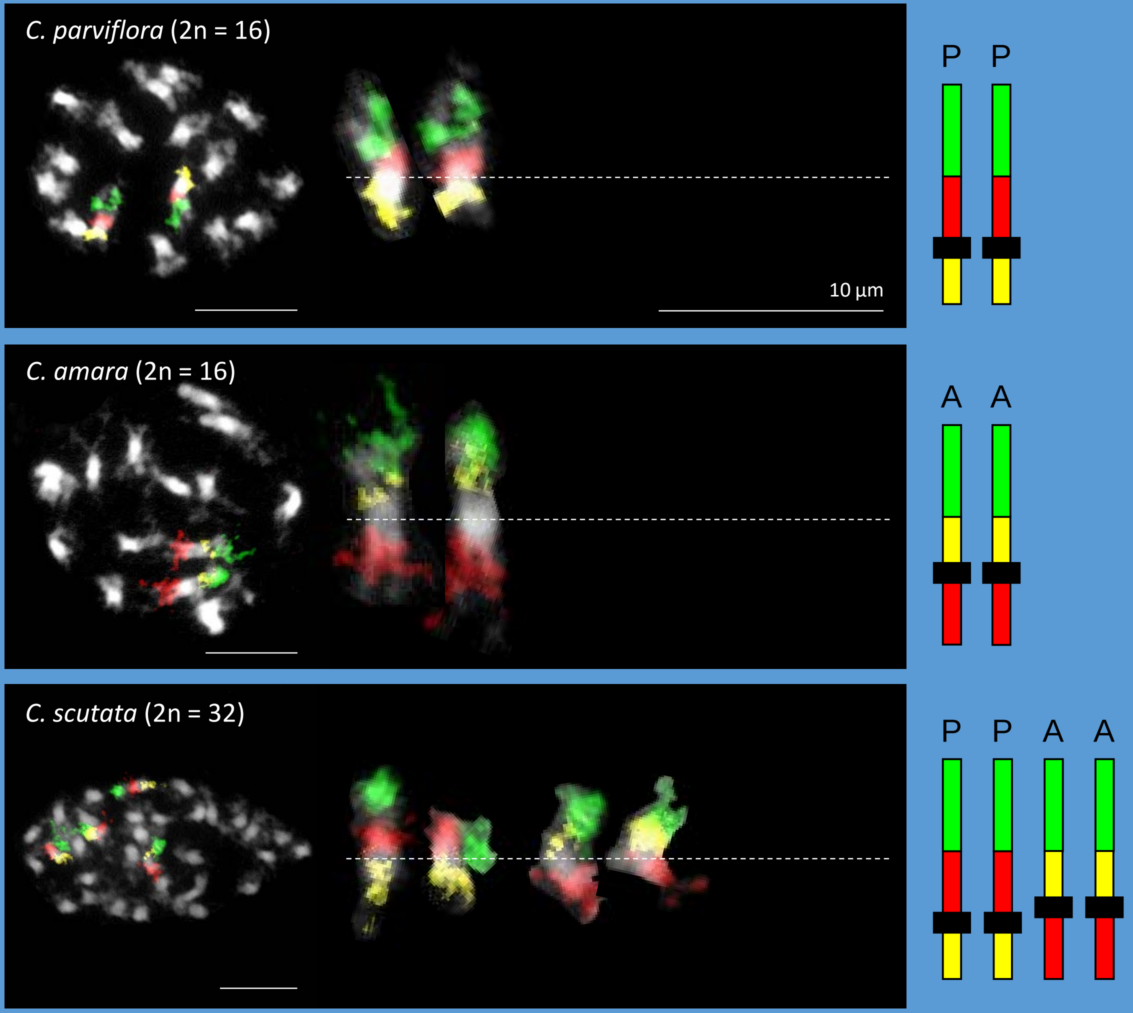
Origin of European *Cardamine flexuosa*

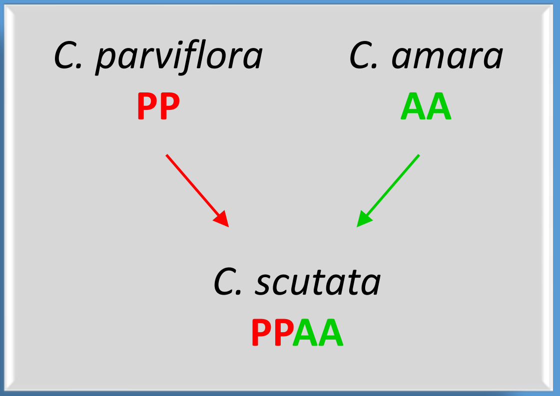


Both rearranged homeologues have undergone an identical **pericentric inversion followed by a reciprocal translocation with breakpoints within both genomic blocks I**, exchanging unequal proportions of the upper arms of progenitor chromosomes CA4 and CH4. Both chromosomes shared altered collinearity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

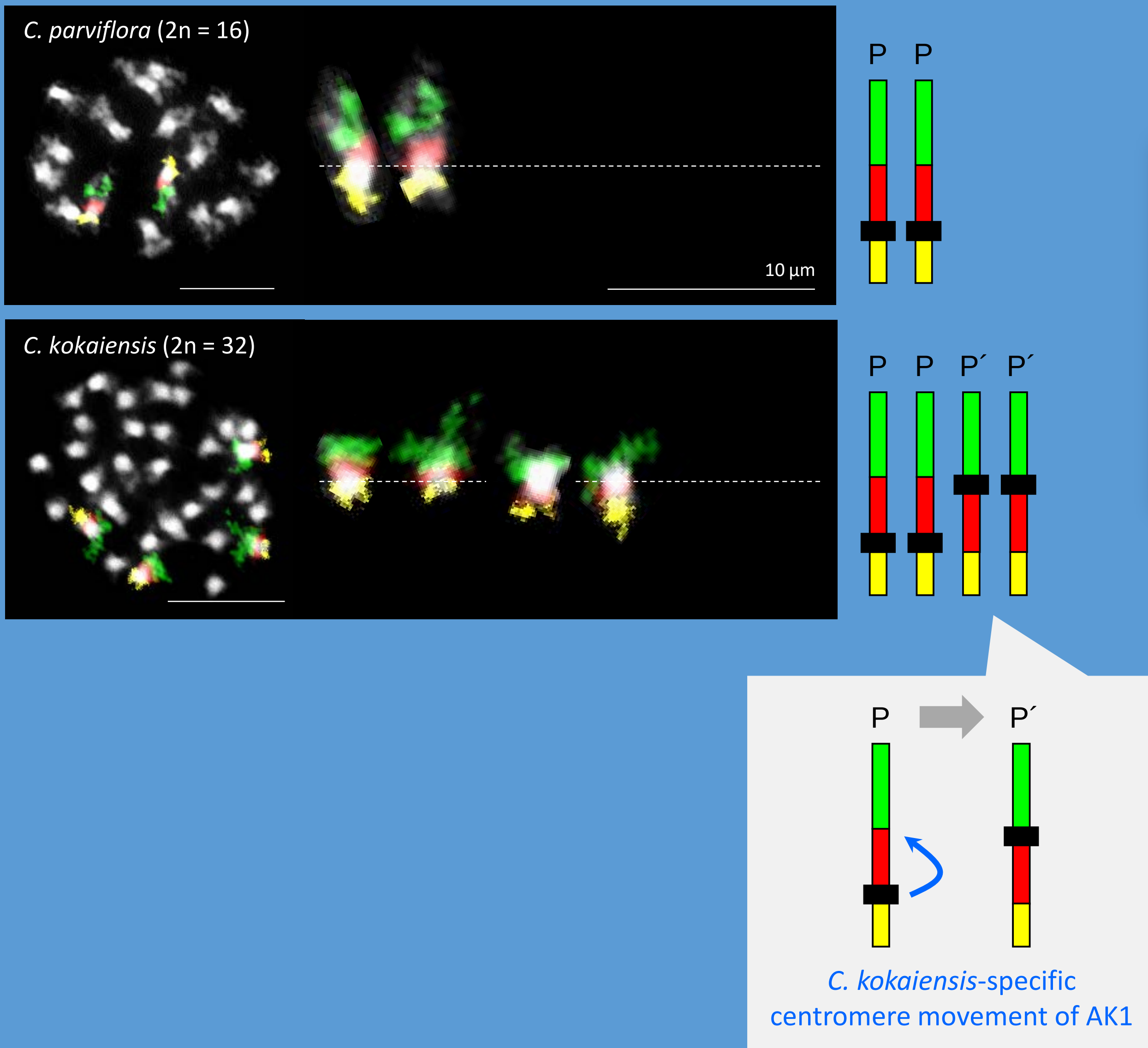
Mandáková, Marhold & Lysák, 2014, New Phytologist 201: 982–992.

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



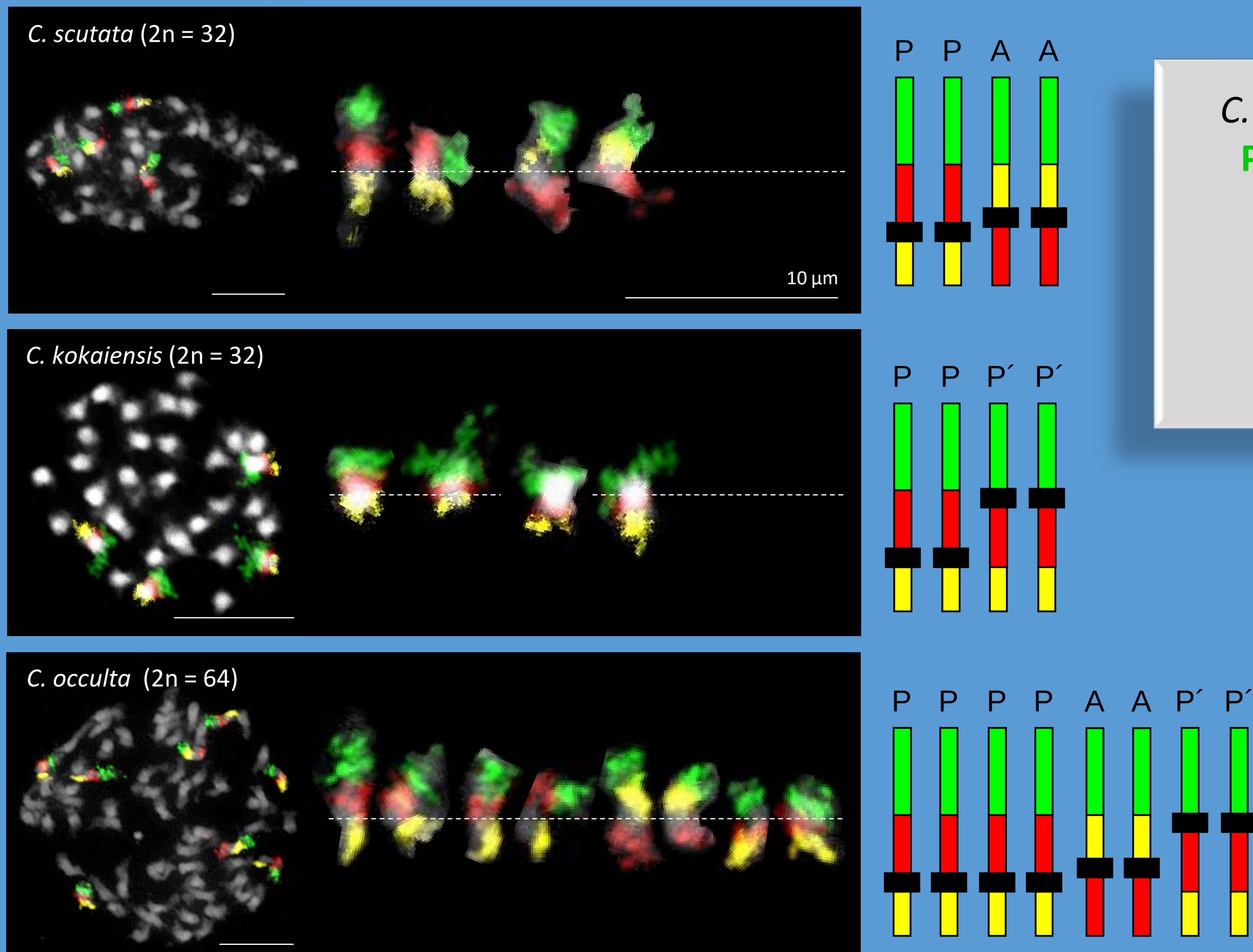


Cardamine kokaiensis (2n = 4x = 32)



C. parviflora
PP
↓
C. kokaiensis
P P P´P´

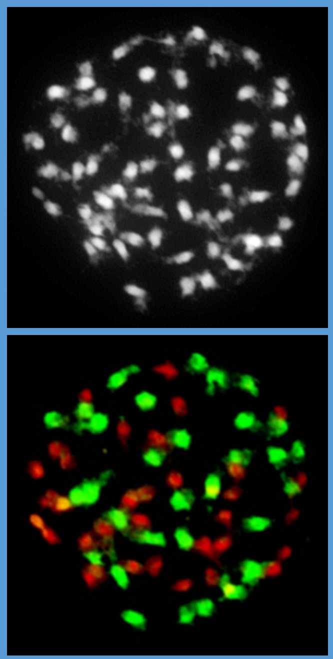
Cardamine occulta (2n = 8x = 64)



C. scutata — P P A A

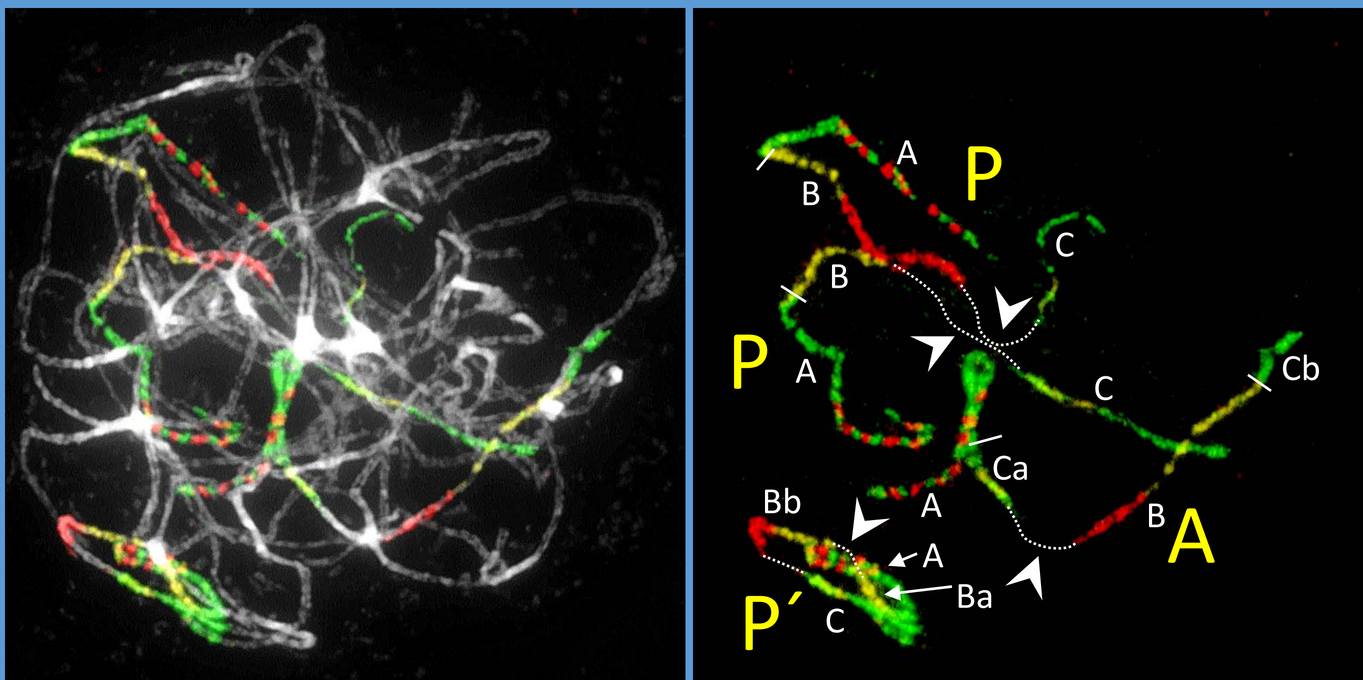
C. kokaiensis — P P P´P´

C. occulta — P P P P A A P´P´



Cardamine occulta (2n = 8x = 64)

Fine-scale rearrangements investigated on pachytene (meiotic) chromosomes:

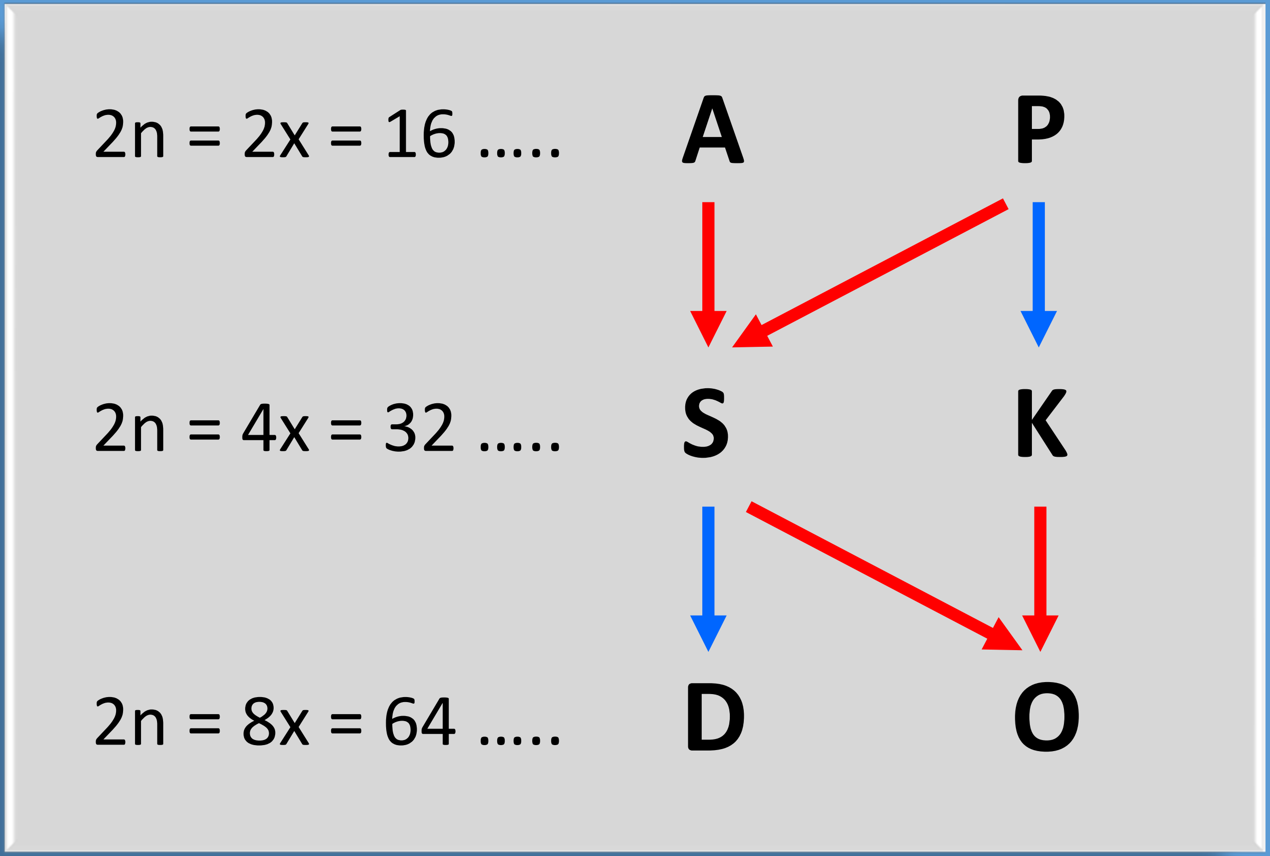


C. kokaiensis (PP´)

P P A P´

C. scutata (PA)

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*