



Od spor po sporofyty: využití průtokové cytometrie v biosystematice kaprad'orostů

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Jsou kaprad'orosty něčím výjimečné?

v zásadě NE

- průtoková cytometrie má u kaprad'orostů v zásadě stejné uplatnění jako u dalších skupin rostlin
- hodnocení velikosti genomu u **výtrusů**, autonomních **gametofytů** a nejběžněji ze **sporofytů**

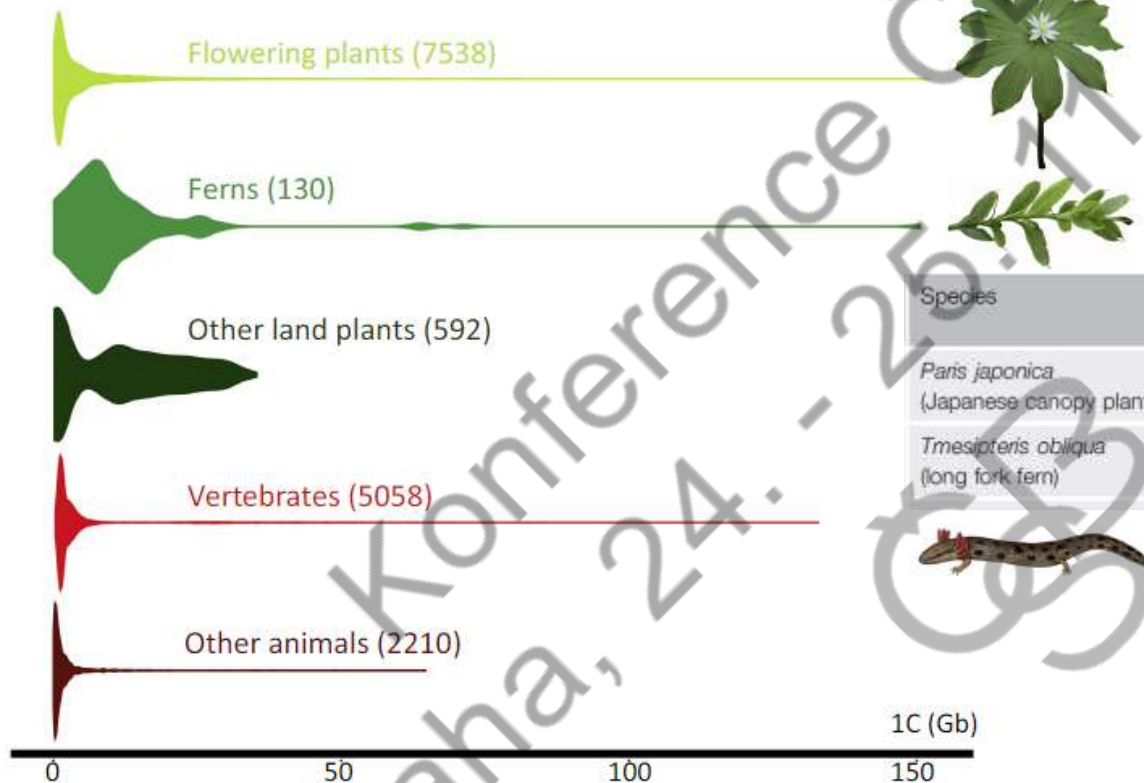


- u řady rodů (*Cystopteris*, *Huperzia*) lze analyzovat tkáně vysušené silikagelem se stejným výsledkem jako u živých tkání



Rozsah velikosti genomu

v průměru spíše velké
genomy
až gigantické genomy



Species	GS ¹	Method ²	2n ³	Ploidy ⁴
<i>Paris japonica</i> (Japanese canopy plant)	148.8	FC:PI	40	8
<i>Tmesipteris obliqua</i> (long fork fern)	147.3	FC:PI	416	8

Monilophyta

1C=13,6 (0,77-147,3)

Lycopodiophyta 1C=3,8 (0,09-12)

Figure 1. Violin Plots Showing the Frequency and Range of Genome Sizes in Different Eukaryote Groups, Together with Illustrations (Right) for Some of the Species with the Largest Genome Sizes. From top to bottom, *Paris japonica* (1C = 148.8 Gb), *Tmesipteris obliqua* (1C = 147.3 Gb), *Protopterus aethiopicus* (1C = 130.0 Gb), and *Necturus lewisi* (1C = 118.0 Gb). Data are taken from the Plant DNAC-values Database (<http://data.kew.org/cvalues/>), the Animal Genome Size Database (www.genomesize.com), and published data not yet included in these databases. Numbers in brackets following eukaryotic group names refer to the number of genome size estimates incorporated in each plot. Photographs from the top: Wikimedia commons/Maarten Christenhusz/Wikimedia commons/Joseph E. Trumpey.

Trends in Plant Science

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Trends in Plant Science

CellPress

Opinion

Is There an Upper Limit to
Genome Size?

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Harald Schneider,^{3,4} Andrew R. Leitch,⁵ and Ilia J. Leitch^{1,4}

Evoluce genomu u kapradin

- velikost genomu známa pro 2,8% druhů
- celogenomové duplikace (WGD) se vyskytují v celém systému kapradin
- korelace velikosti genomu s počtem chromozomů

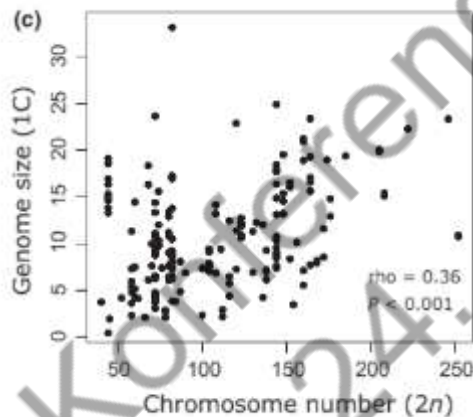


Fig. 2 Correlation plots of chromosome number ($2n$; x-axis) vs genome size ($2C$; y-axis). Each dot corresponds to one accession. Linear regression statistics are given by rho and P-values. (a) Plot including all ferns; (b) plot including all nonleptosporangiate fern orders; (c) plot including all leptosporangiate fern orders; (d) plot including leptosporangiate fern orders without Osmundales.

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Genome evolution of ferns: evidence for relative stasis of genome size across the fern phylogeny

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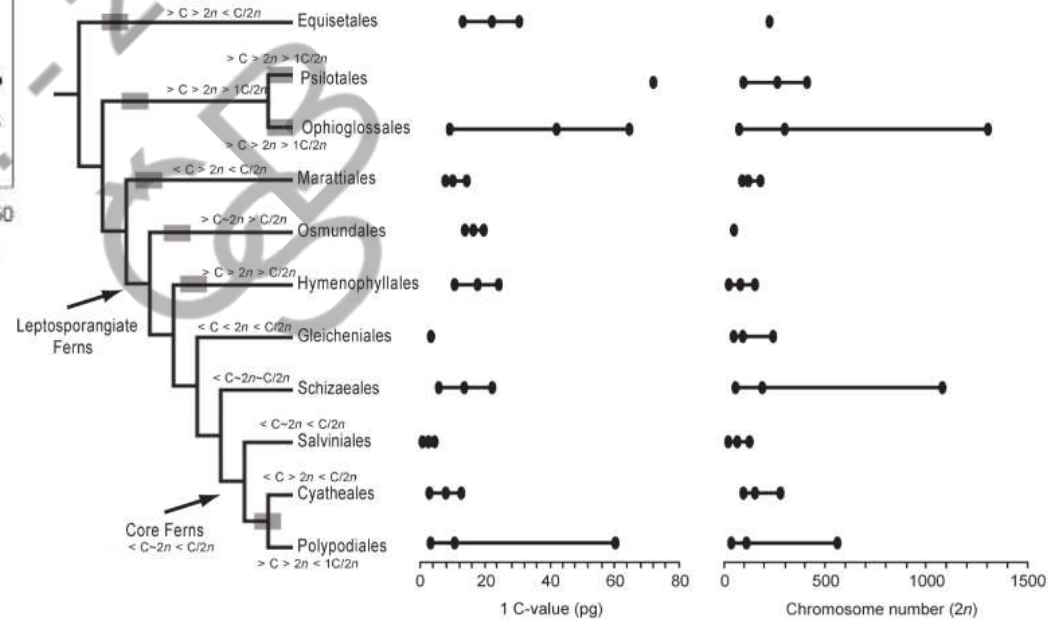


Fig. 1 Summary of the observed distribution of genome size and chromosome number variation among the 11 orders of ferns. Bars indicate the range of variation with the maximum, mean and minimum values indicated via dots. The phylogeny summarizes the currently accepted hypothesis with the horsetails considered sister to the remaining fern clade (for alternative topologies, see Supporting Information Fig. S2). Terminal taxa correspond to orders according to Smith *et al.* (2006) and Christenhusz *et al.* (2011), whereas leptosporangiate ferns and core ferns are marked by arrows. Based on the data given in Tables 1 and 3, some clades show notable trends such as increase (>)/decrease (<) of genome size ($1C$), increase of chromosome numbers ($2n$), or changes in chromosome size ($2C/2n$). Grey squares indicate clades that show some evidence for whole-genome duplications.

Od spór



Fig. 4 Histograms showing the relative DNA contents of fern and lycophyte spore and leaf nuclei obtained using flow cytometry. The black, gray, and white arrows indicate spore nuclei, leaf nuclei, and $2n$ nuclei of *Nicotiana tabacum* 'Xanthi', respectively.

New Phytologist (2017) 213: 1974–1983
doi: 10.1111/nph.14291

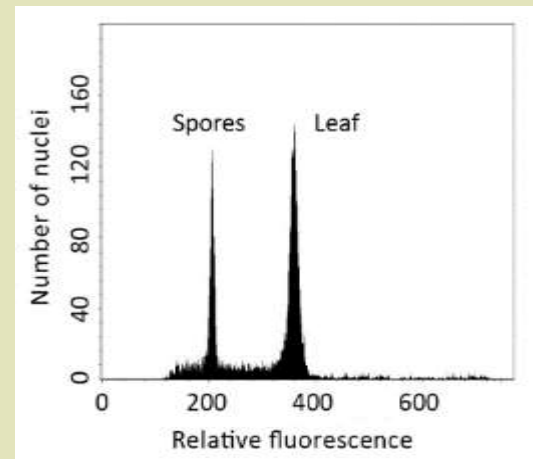
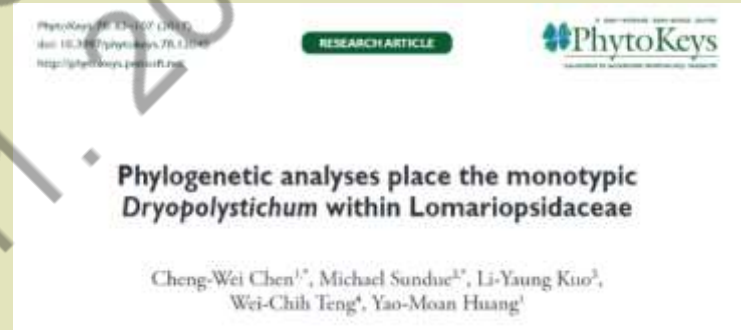
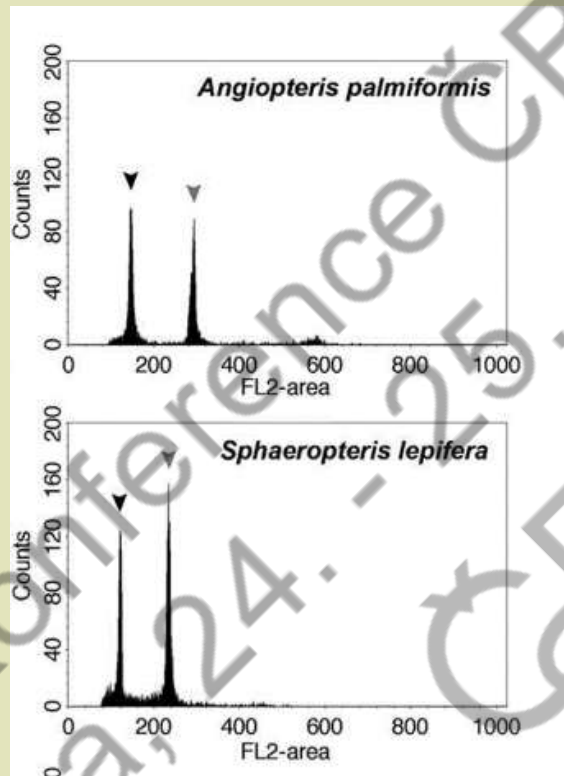


Figure 5. Relative DNA contents of *Dryopolystichum phaeoglossum* spore and leaf nuclei inferred by flow cytometry.

Methods

Methods

Evaluating the spore genome sizes of ferns and lycophytes: a flow cytometry approach

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Taiwan; ⁵Division of Botanical Garden, Taiwan Forestry Research Institute, Taipei 10006, Taiwan; ⁶Dr. Cuiqin Ren Botanic Conservation and Environmental Protection Foundation,

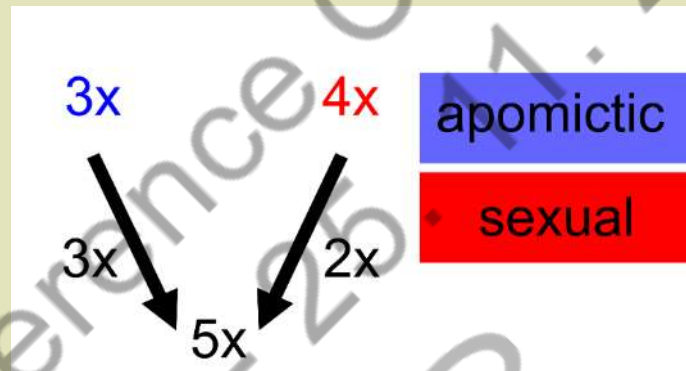
Guangdong Province 510646, Taiwan



Cystopteris fragilis:
potvrzení neredukovaných
výtrusů u 4x a 5x puchýřníku
(optimalizace metody)

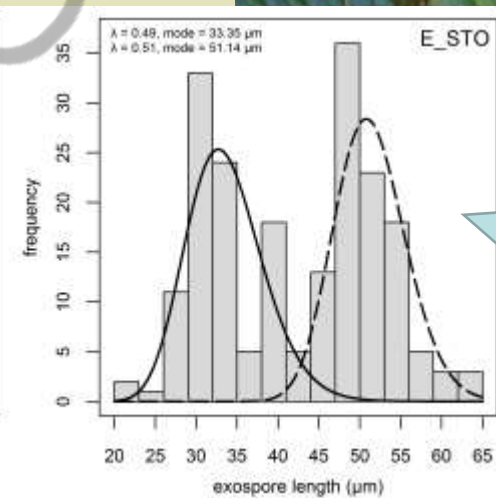
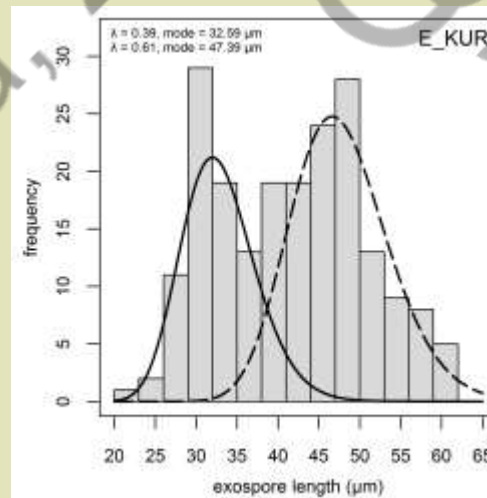
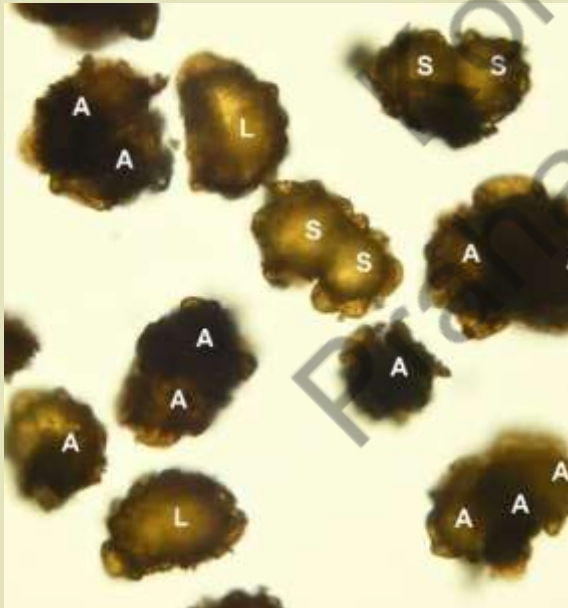
.... přes gametofyty ...

D. borrieri × *D. filix-mas*



A = abortované
S = malé
L = velké

Dryopteris ×critica





The diagram illustrates the evolutionary pathways of *Dryopteris x critica*. At the top, two pathways are shown: an apomictic pathway (blue box) and a sexual pathway (red box). The apomictic pathway starts with a 3x ancestor, leading to a 3x intermediate, and then to a 5x descendant. The sexual pathway starts with a 4x ancestor, leading to a 2x intermediate, and then to a 5x descendant. The 5x descendant is labeled *Dryopteris x critica*. Below this, three arrows point from the 5x descendant to three boxes labeled A, 5x, and 2.5x, representing further evolutionary outcomes.

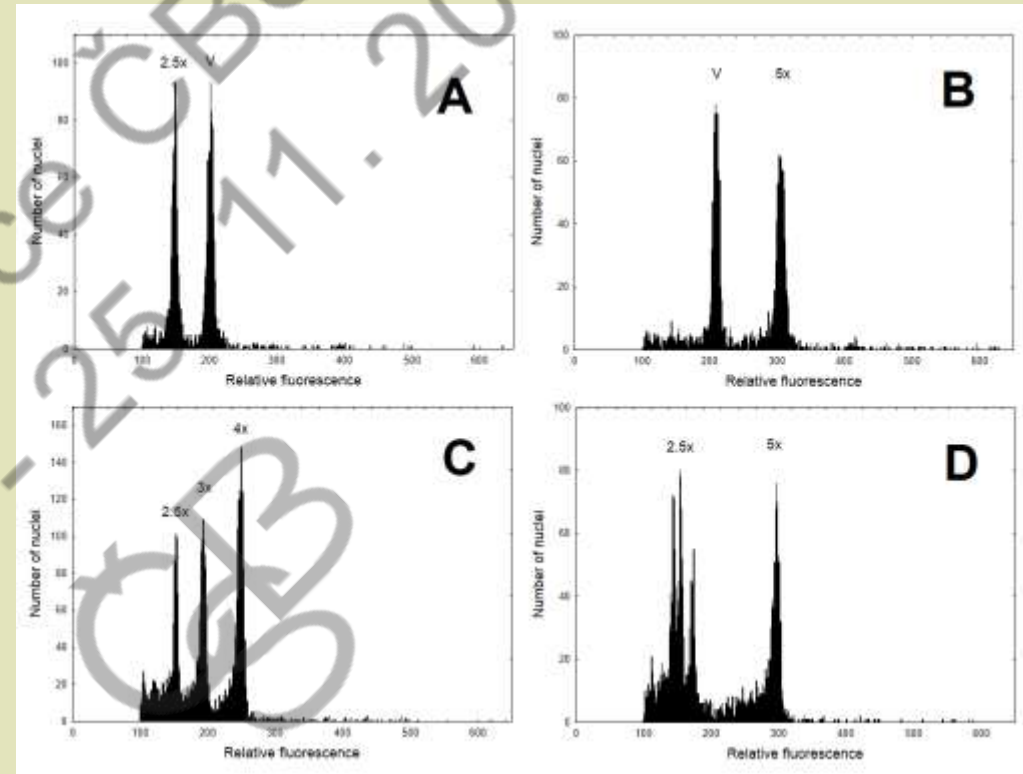
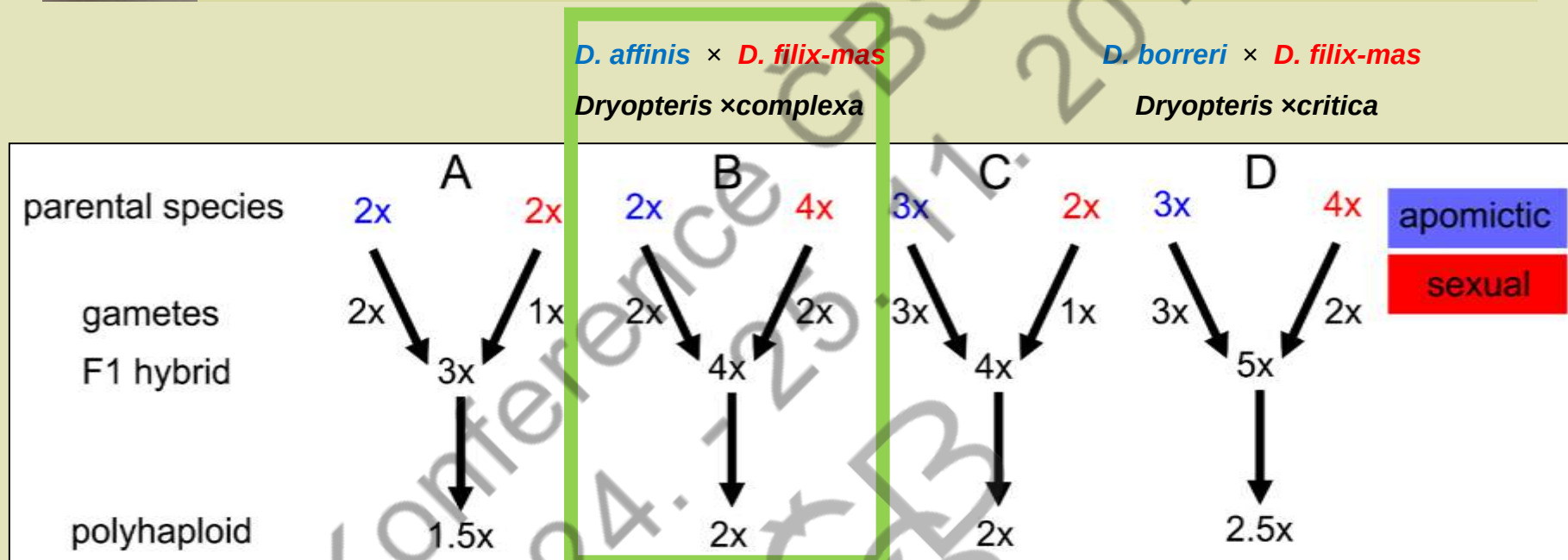


Fig. 4. Flow cytometric profiles (DAPI staining) of F_2 gametophytes of pentaploid *Dryopteris* $\times$ *critica*: (A) 2.5x gametophyte and apogamous sporophyte analysed with the internal standard *Vicia faba*; (B) 5x gametophyte and apogamous sporophyte analysed with the internal standard *V. faba*; (C) simultaneous analysis of 2.5x F_2 gametophyte of *D. \times critica*, triploid *D. borrieri* and tetraploid *D. filix mas* (both with known chromosome count; Ekrt *et al.*, 2009); (D) simultaneous analysis of three 2.5x gametophytes and one 5x gametophyte arisen from one maternal plant (E_STO) – three separate peaks of 2.5x gametophytes are clearly visible, corroborating variation in the genome size between the gametophytes.



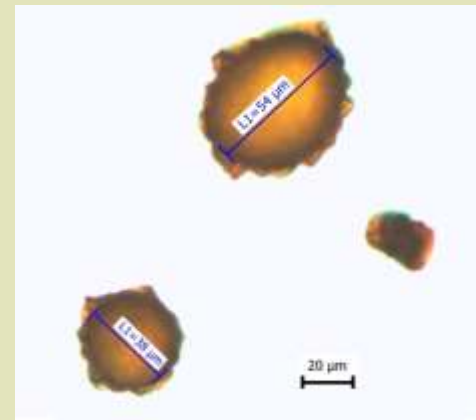
.... přes gametofyty ...



Teoretické schéma křížení sexuálních a apomiktických druhů kapradin a tvorba polyhaploidů



Dryopteris × *complexa* (4x)
Německo (Schwarzwald)





.... přes gametofyty ...

Cystopteris fragilis: studium
ploidně smíšených populací



4x a 5x vytváří variabilní
reprodukční strategie

6x vytváří uniformní
potomstvo

4x sporofyt



Heteroploidní hybridizace?

- nízká (0,59 %)



.... přes gametofyty ...

- jak se rozmnožují pentaploidi puchýřníku křehkého?



záleží na populaci

Lokalita:	Roztoky	Oheb	Bělá
gametofyty	2x, 3x	2x, 3x, 4x, 5x	5x
reprodukce	pohlavní klonální	pohlavní apomixie	apomixie
SAI	96,16 %	99,10 %	94,66 %

I přes velmi nízkou fertilitu je 5x puchýřník schopný vyprodukovat pár životaschopných gametofytů





zapomeňte na homosporii!



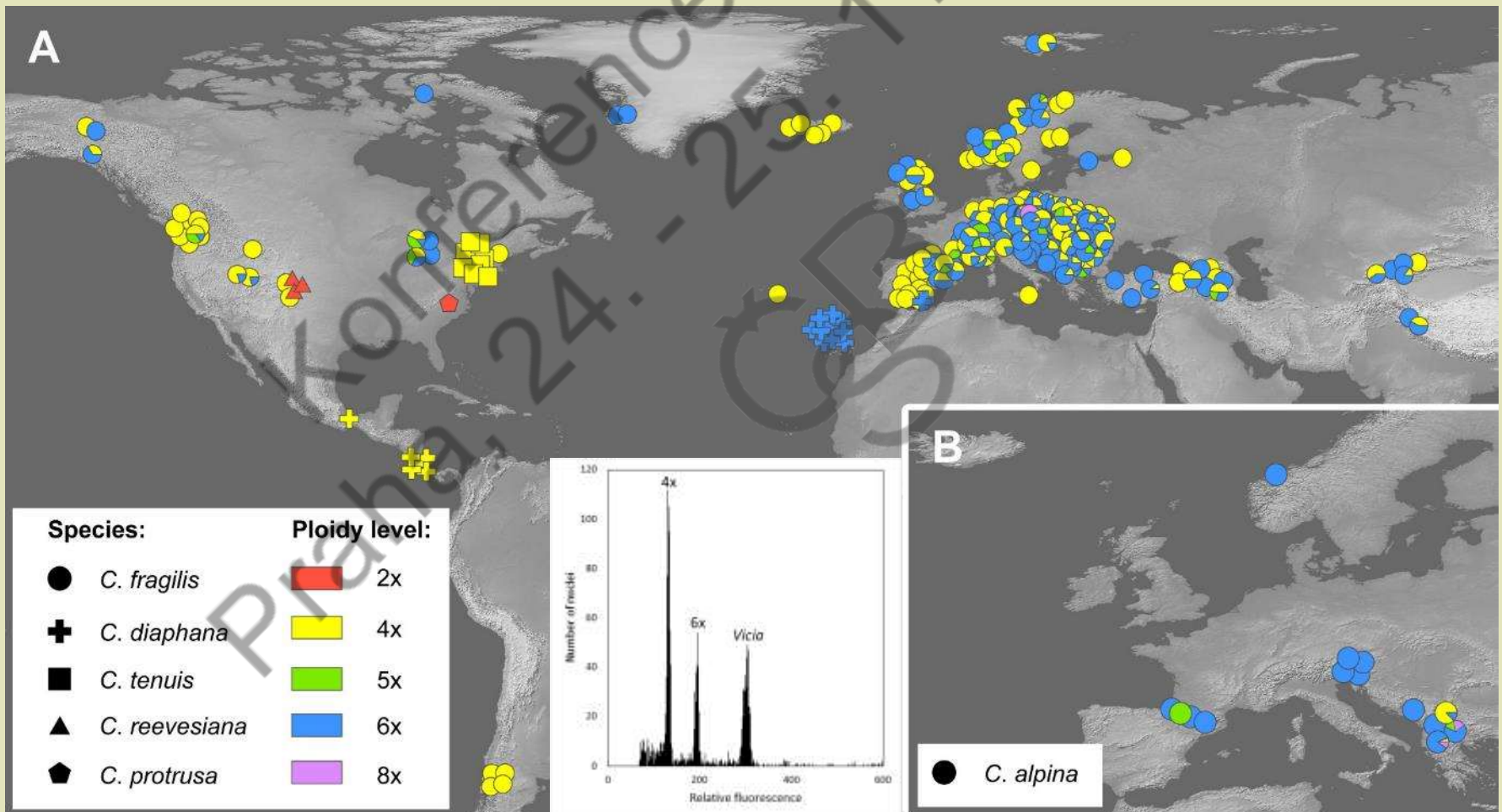
blíží se „krypto-heterosporie“ !!



.... po sporofyty

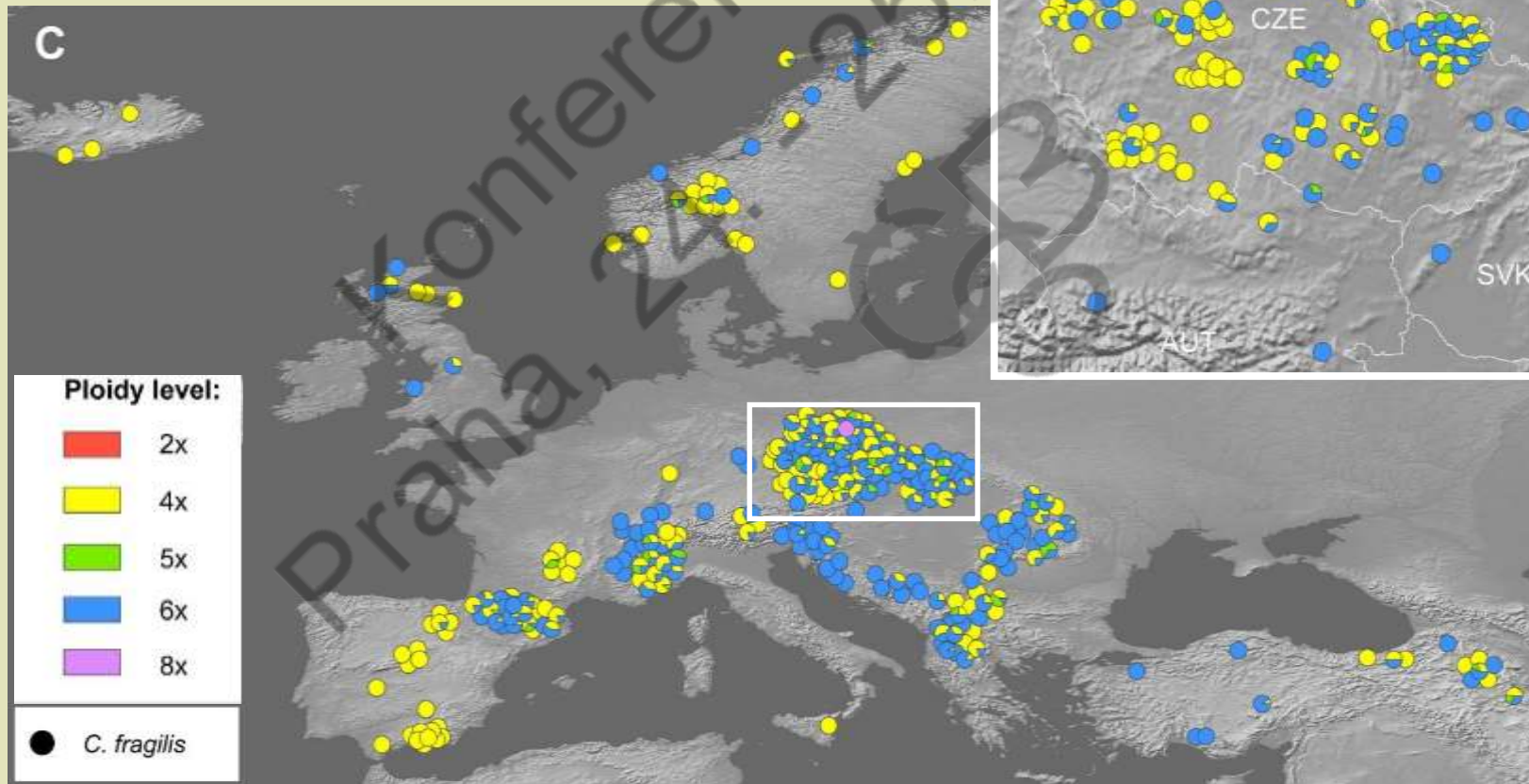


studium komplexu puchýřníku křehkého - *Cystopteris fragilis*
-nejrozsáhlejší cytogeografie kapradin (5518 rostlin/449 lokalit)



.... po sporofyty

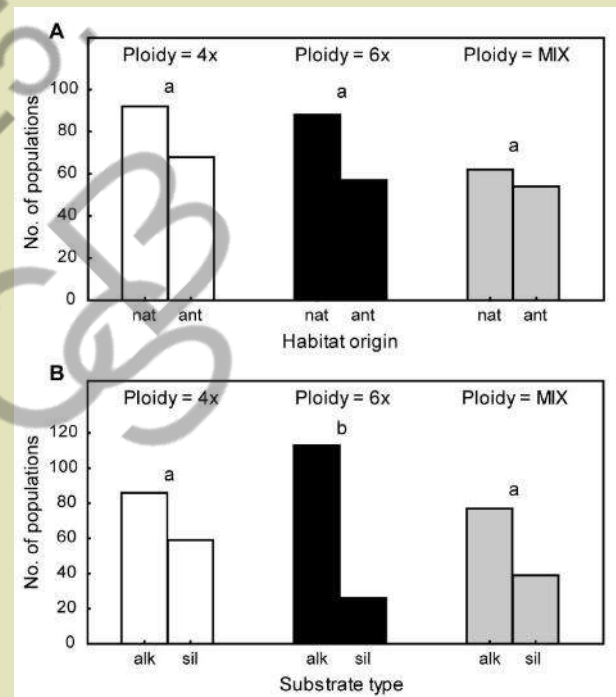
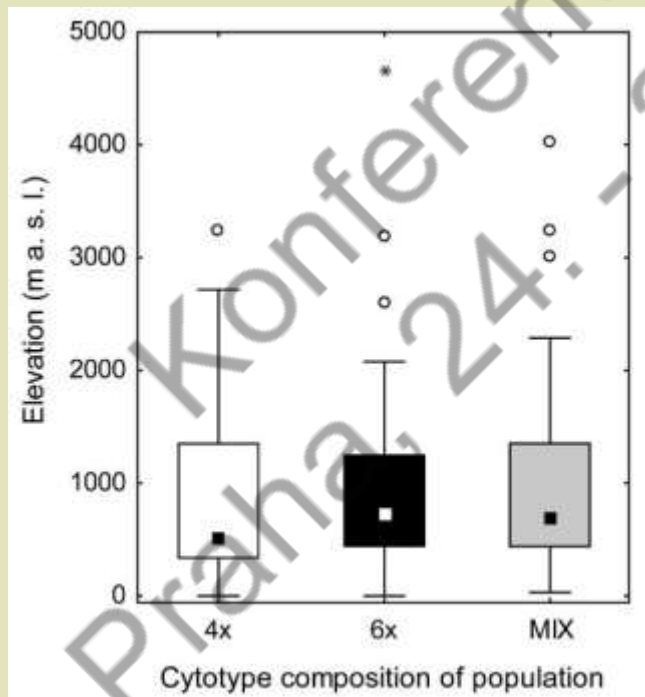
- převládají 4x a 6x
- ve 40% populací tyto cytotypy koexistují a mohou tvořit hybridní 5x



.... po sporofyty



-ekologické podmínky (nadmořská výška, typ substrátu, primární/sekundární [zdi] výskyt) nehrají roli v separaci jednotlivých cytotypů



Hanušová K., Čertner M., Urfus T., Koutecký P., Košnar J., Rothfels C. J., Jarolímová V., Ptáček J., Ekt L. (2018): Widespread co-occurrence of multiple ploidy levels in fragile ferns (*Cystopteris fragilis* complex; Cystopteridaceae) likely stems from similar ecology of cytotypes, their efficient dispersal and inter-ploidy hybridization. – *Annals of Botany* (in press.)

.... po sporofyty

-studium „známých“ skupin
= *Dryopteris carthusiana* agg.



podrobnější vhled do
mechanismů
homo/heteroploidní frekvence
hybridizace v přírodě

Botany

American Journal of Botany 97(7): 1205–1219, 2010.

SPECIES BOUNDARIES AND FREQUENCY OF HYBRIDIZATION IN
THE *DRYOPTERIS CARTHUSIANA* (DRYOPTERIDACEAE) COMPLEX:
A TAXONOMIC PUZZLE RESOLVED USING GENOME SIZE DATA

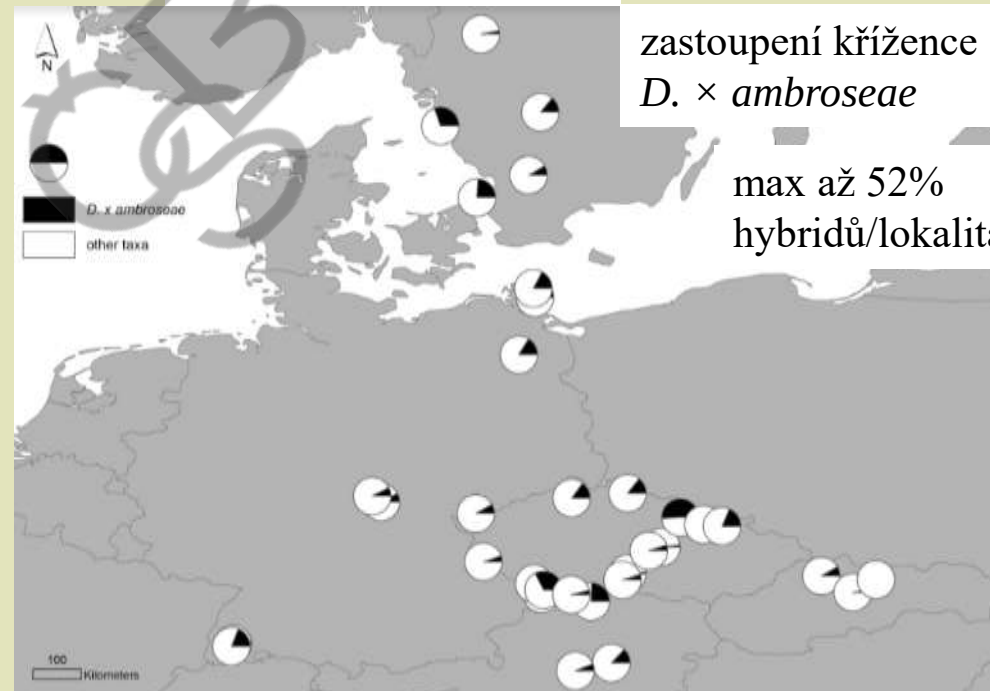
LIBOR EKR^{1,2,5}, RENATA HOLUBOVÁ³, PAVEL TRÁVNÍČEK^{1,4}, AND JAN SUDA^{3,4}

-100 jedinců na lokalitu
-determinace pomocí FCM

zastoupení křížence
D. × ambroseae

max až 52%
hybridů/lokalita

Taxon	Počet	Podíl
<i>Dryopteris carthusiana</i>	678	16.6%
<i>Dryopteris dilatata</i>	1791	43.8%
<i>Dryopteris expansa</i>	1158	28.3%
<i>Dryopteris x sarvelae</i>	0	0%
<i>Dryopteris x deweveri</i>	21	0.5%
<i>Dryopteris x ambroseae</i>	441	10.8%
Total	4089	100%





.... po sporofyty

studium dosud neprobádaných skupin



Huperzia selago agg.

stávající představa:

Huperzia selago
(polyploid)



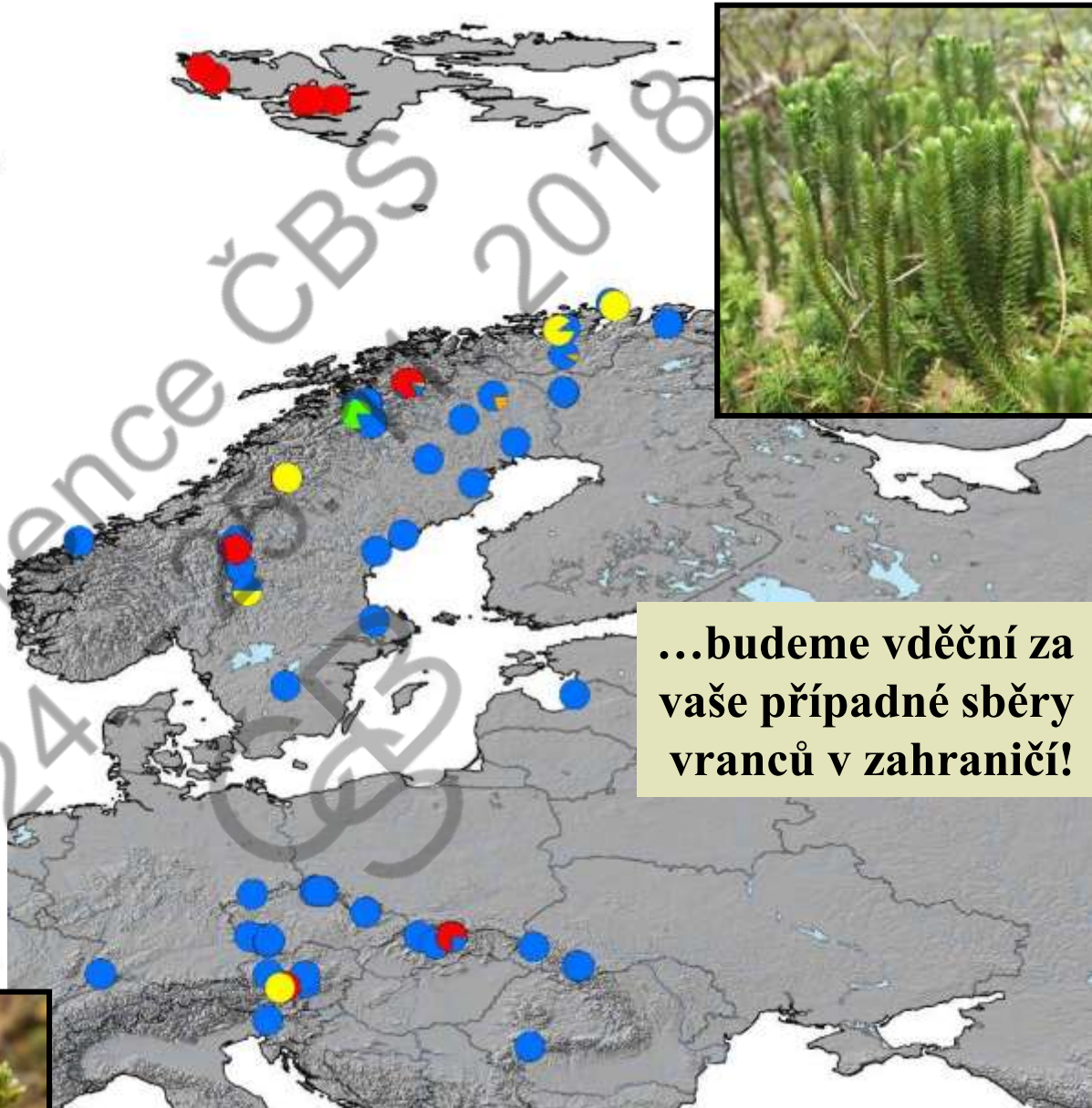
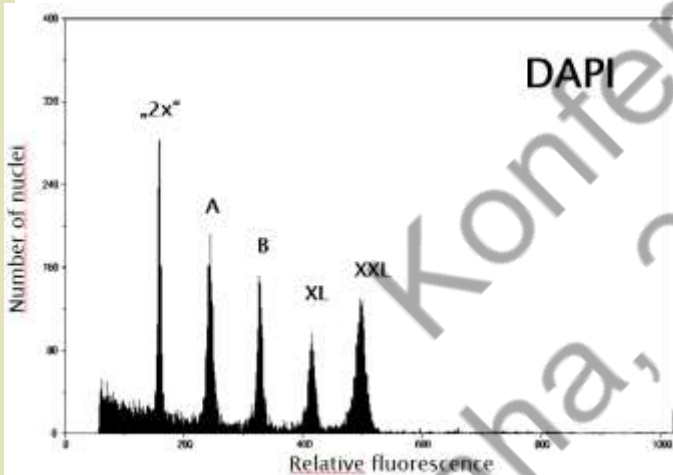
Huperzia arctica
(diploid)



Foto: W. Testo



-  A = alpský cytotyp
-  odchylka od běžného cytotypu
-  B = běžný cytotyp
-  XL = velký cytotyp
-  XXL = největší cytotyp



**...budeme vděční za
vaše případné sběry
vranců v zahraničí!**



... děkujeme za pozornost