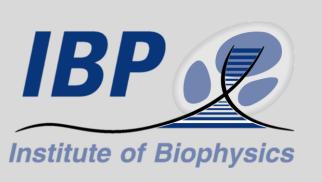


How to study common hop (*Humulus lupulus* L.) karyotype evolution: genomic and cytogenetics approaches Horáková Lucie¹, Bačovský Václav¹, Čegan Radim¹, Janoušek Bohuslav¹, Hubinský Marcel¹, Patzak Josef², Hobza Roman¹ ¹ Department of Plant Developmental Genetics, Institute of Biophysics of the Czech Academy of Sciences, Kralovopolska 135, 612 65 Brno, Czech Republic.

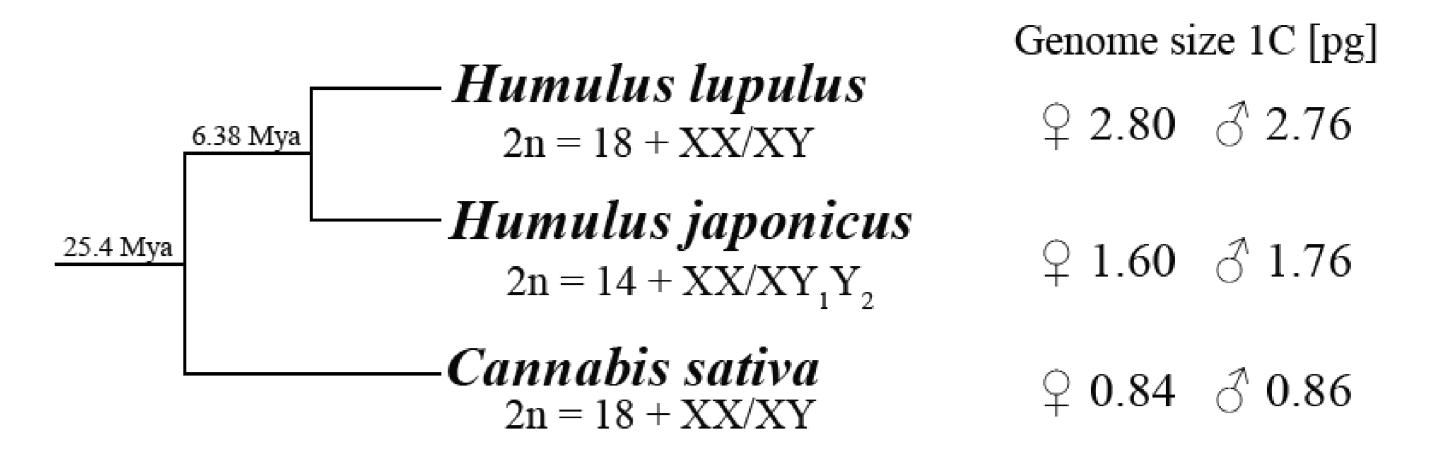


² Hop Research Institute Co. Ltd, Kadaňská 2525, 438 46 Žatec, Czech Republic.

Introduction

- Humulus lupulus (common hop) is a perennial and dioecious plant with XX/XY • chromosome system. Sex chromosomes are heteromorphic – the Y chromosome is smaller compared to X chromosome.
- Related species Humulus japonicus and Cannabis sativa differ in genome size, • chromosome number, and sex chromosome system. Nevertheless, it is a unique model for studying the evolution of sex chromosomes.
- Despite hop's importance in the brewing industry evolutionary processes shaping the genomic • landscape in the hop are still poorly understood.

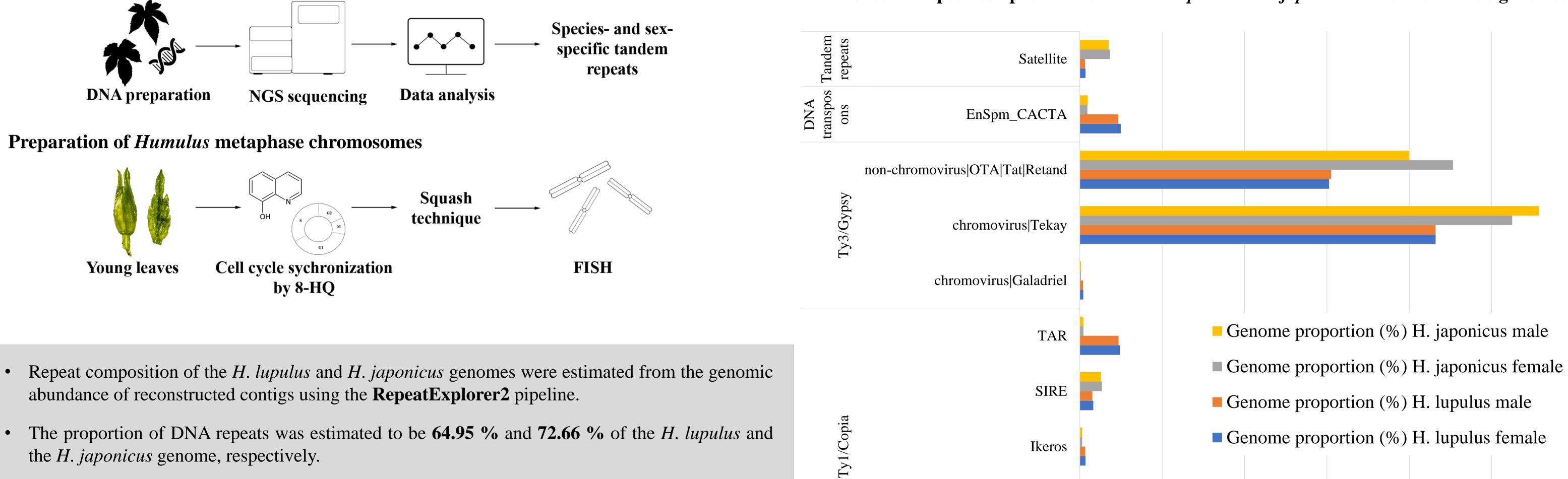
The phylogenetic relations in *Cannabaceae* family



Comparative analysis of *Humulus lupulus* and *Humulus japonicus*

Identification of DNA repeats

Differences in repeat composition between *H. lupulus* or *H. japonicus* female and male genomes



- The proportion of DNA repeats was estimated to be 64.95 % and 72.66 % of the *H*. *lupulus* and • the *H. japonicus* genome, respectively.
- Minimal differences between female and male genomes of both species were observed. Considerable differences were found for class of repats Ty1/Copia (Angela, SIRE, and TAR), Ty3/Copia (Tekay, Athila, and Retand), and tandem repeats (more in the graph).
- This study is focused on the distribution of tandem repeats (satellites) which represent 0.35 % • of the *H*. *lupulus* genome and **1.80** % of the *H*. *japonicus* genome.

Distribution of tandem repeats on autosomes and sex chromosomes

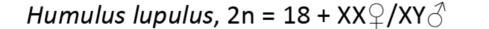
HSR HSR0

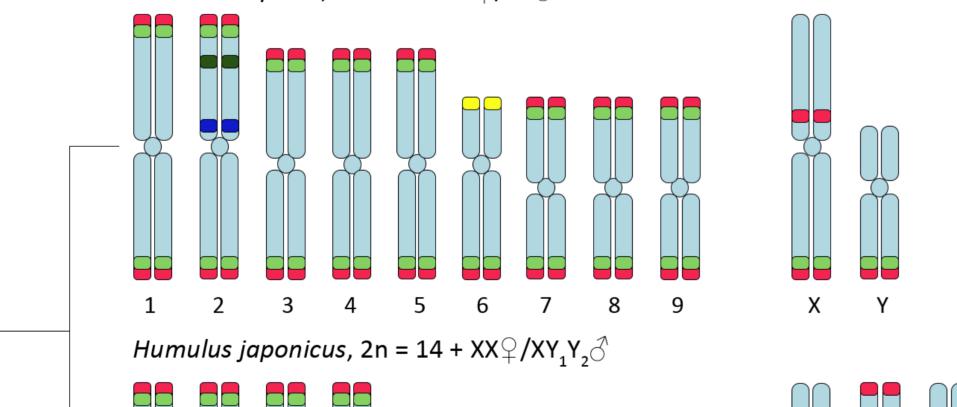
 Cluster 293 • 5S rDNA • 45S rDNA

Cluster 228 Cluster 179

- Young leaves of *H. lupulus* (Saaz hop) and *H. japonicus* treated by 8-hydroxyquinoline (8-HQ) were used for chromosomes preparation. 8-HQ increases the number of cell in mitosis and allowing to metaphase chromosomes isolation.
- Tandem repeats HSR¹, HSR0², and newly identified cluster cl293 were localized on *H. lupulus* chromosomes (HSR and HSR0 in subtelomeric regions and cl293 autosome 2 specific pericentromeric signal).
- Positions of tandem repeats HJSR³, newly identified clusters cl228, cl179, and cl165 were localized on *H. japonicus* chromosomes (HJSR and cl288 in subtelomeric regions, cl179 and cl165 in pericentromeric regions).
- HSR and HJSR have sequence similarity and have same pattern on metaphase chromosomes. Similar patter were found for HSR0 and cluster 228.









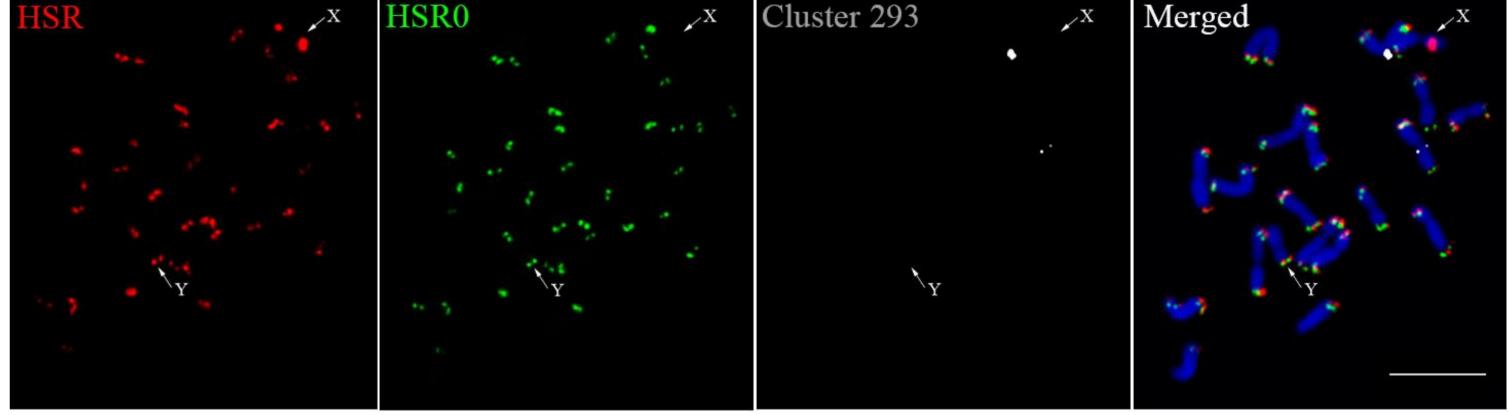
Ikeros

Angela

Ale

0

5



10

Genome proportion (%) H. lupulus female

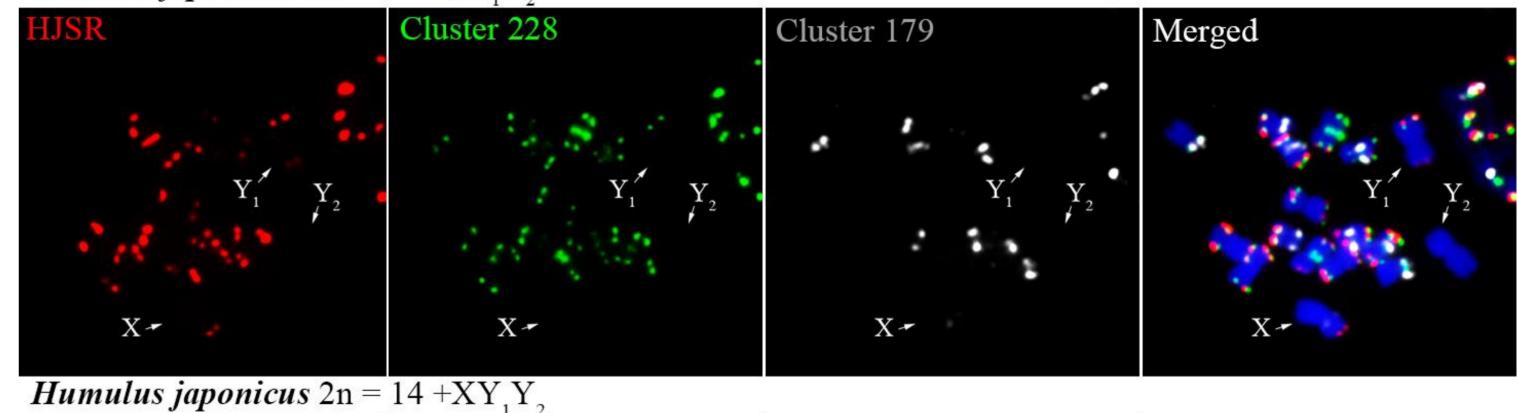
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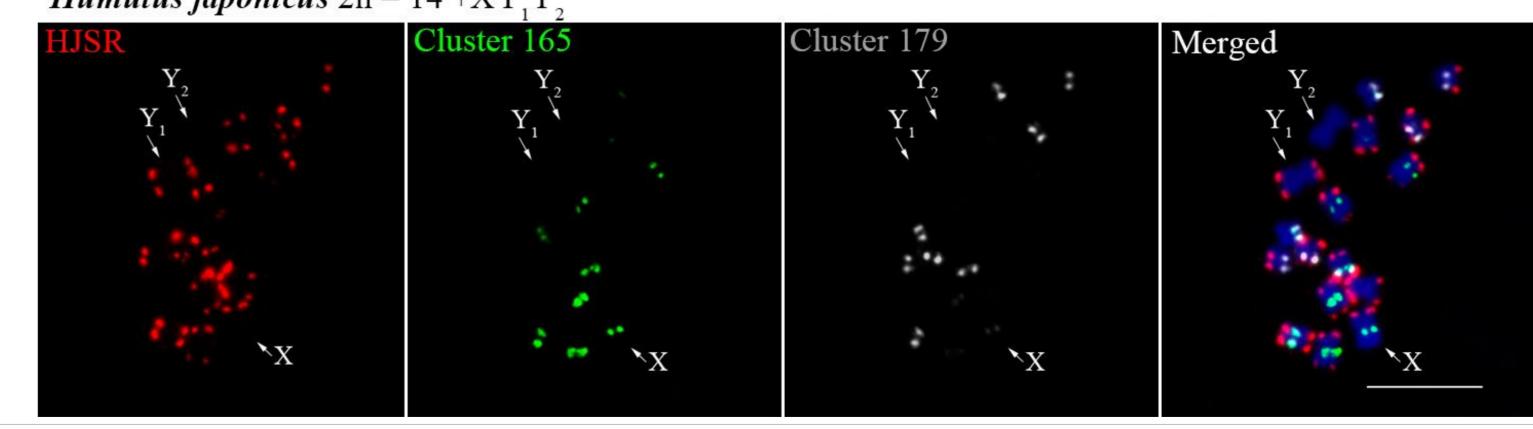
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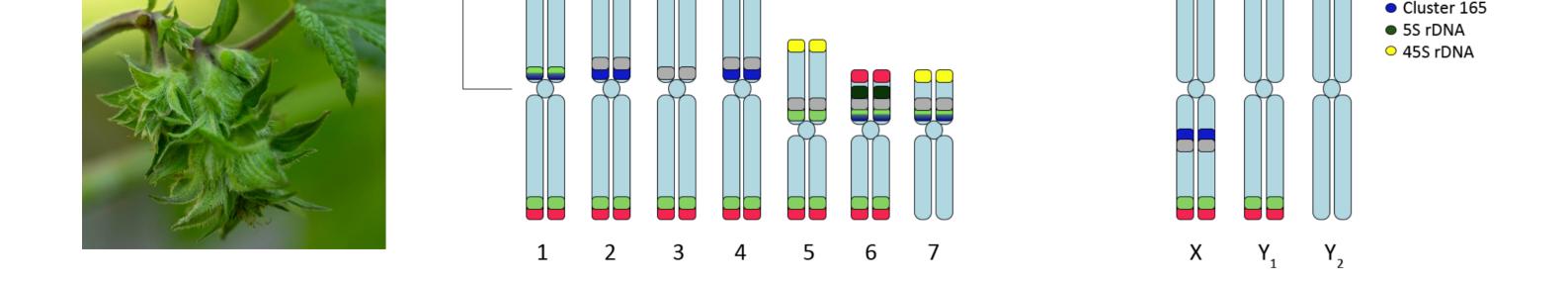
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Humulus japonicus $2n = 14 + XY_1Y_2$







Acknowledgment and References

Conclusion and Future plans

This work was supported by the Czech Science Foundation project No. 22-00301S.

- 1. Divashuk, M. G., Alexandrov, O. S., Kroupin, P. Y., Karlov, G. I. (2011). Molecular cytogenetic mapping of *Humulus lupulus* sex chromosomes. Cytogenetic and Genome Research, 134(3), 213-219.
- 2. Easterling, K. A., Pitra, N. J., Jones, R. J., Lopes, L. G., Aquino, J. R., Zhang, D., Matthews, P. D., Bass, H. W. (2018). 3D molecular cytology of hop (Humulus lupulus) meiotic chromosomes reveals non-disomic pairing and segregation, aneuploidy, and genomic structural variation. Frontiers in plant science, 9, 1501.
- 3. Alexandrov, O. S., Divashuk, M. G., Yakovin, N. A., Karlov, G. I. (2012). Sex chromosome differentiation in Humulus japonicus Siebold & Zuccarini, 1846 (*Cannabaceae*) revealed by fluorescence in situ hybridization of subtelomeric repeat. *Comparative cytogenetics*, 6(3), 239. 4. Kovalchuk, I., Pellino, M., Rigault, P., Van Velzen, R., Ebersbach, J., Ashnest, J. R., Mau, M., Schranz, M. E., Alcorn, J., Laprairie, R. B., McKay, J. K., Burbridge, C., Schneider, D., Vergara, D., Kane, N. C., Sharbel, T. F. (2020). The genomics of Cannabis and its close relatives. Annual Review of Plant Biology, 71, 713-739.
- We identified new cytogenetic markers (cluster cl293, cl228, cl179, and cl165) and localized their position on autosomes and sex chromosomes.
- Our results suggest the occurrence of chromosomal rearrangements during the autosomes and sex chromosome evolution in both species (at least one inversion in *H. lupulus* genome and fusion in *H. japonicus* genome).
 - Our findings provide a new tool to study closely related species of the *Cannabaceae* family and their genome dynamics in relation of their independent XY evolution.