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## Sequencing, annotation, and pangenomics in wheat

Frédéric Choulet

► **To cite this version:**

Frédéric Choulet. Sequencing, annotation, and pangenomics in wheat. Webinaire de l'AFBV, Association Française des Biotechnologies Végétales, May 2022, Clermont-Ferrand, France. hal-04090082

**HAL Id: hal-04090082**

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Submitted on 5 May 2023

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# Sequencing, annotation, and pangenomics in wheat

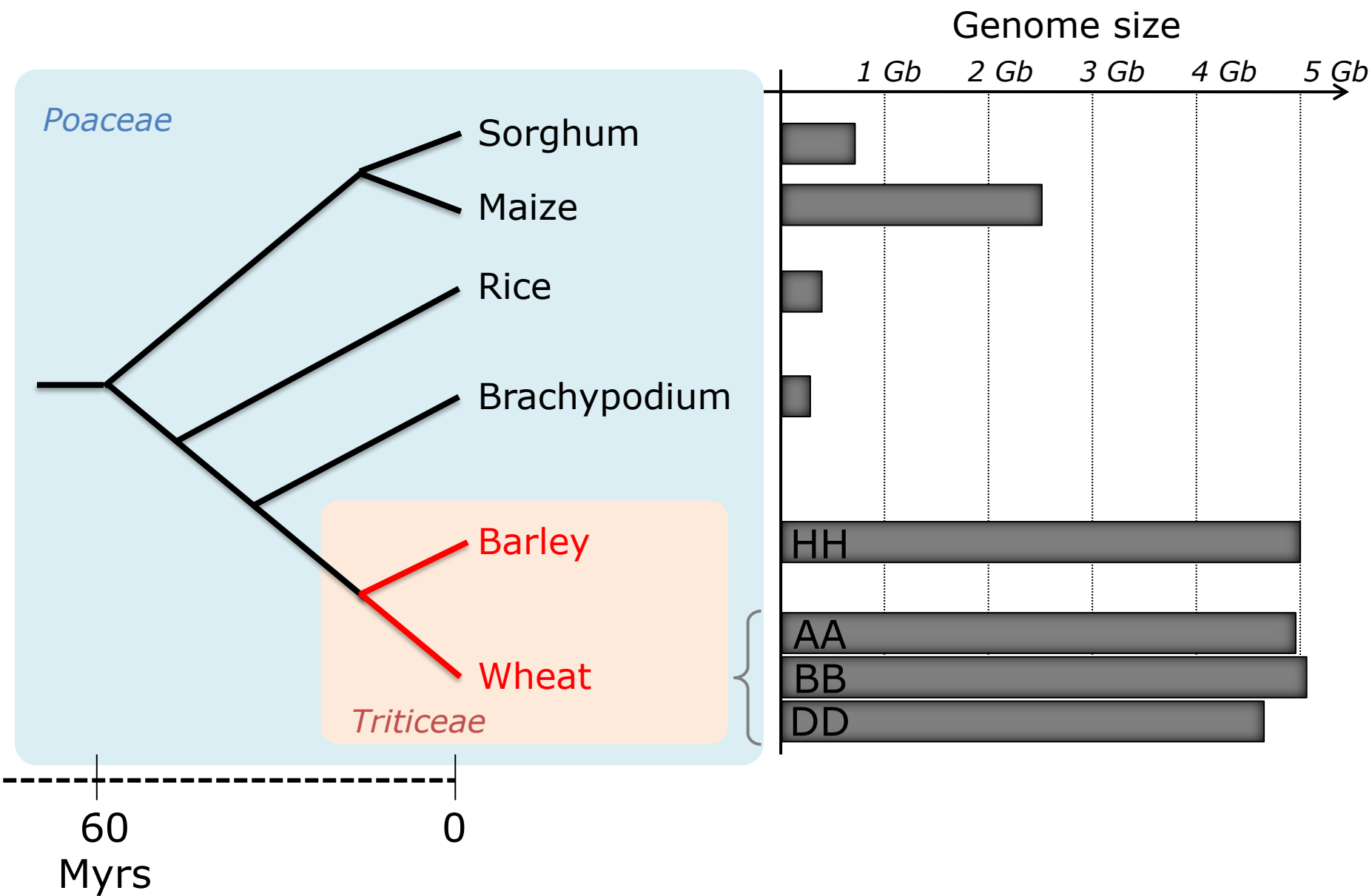
Frédéric Choulet

*GDEC, Clermont-Ferrand, France*

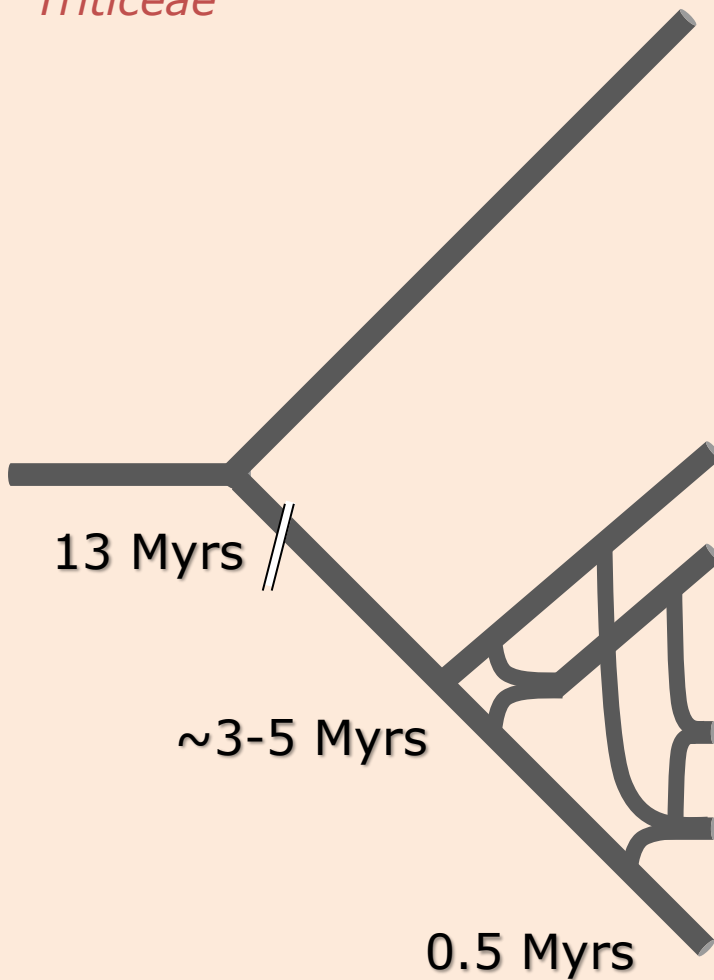


# 1. Introduction

- *Wheat/Triticeae*
- Pangenomics



*Triticeae*



HH *Hordeum*



AA *Triticum urartu*



DD *Aegilops tauschii*

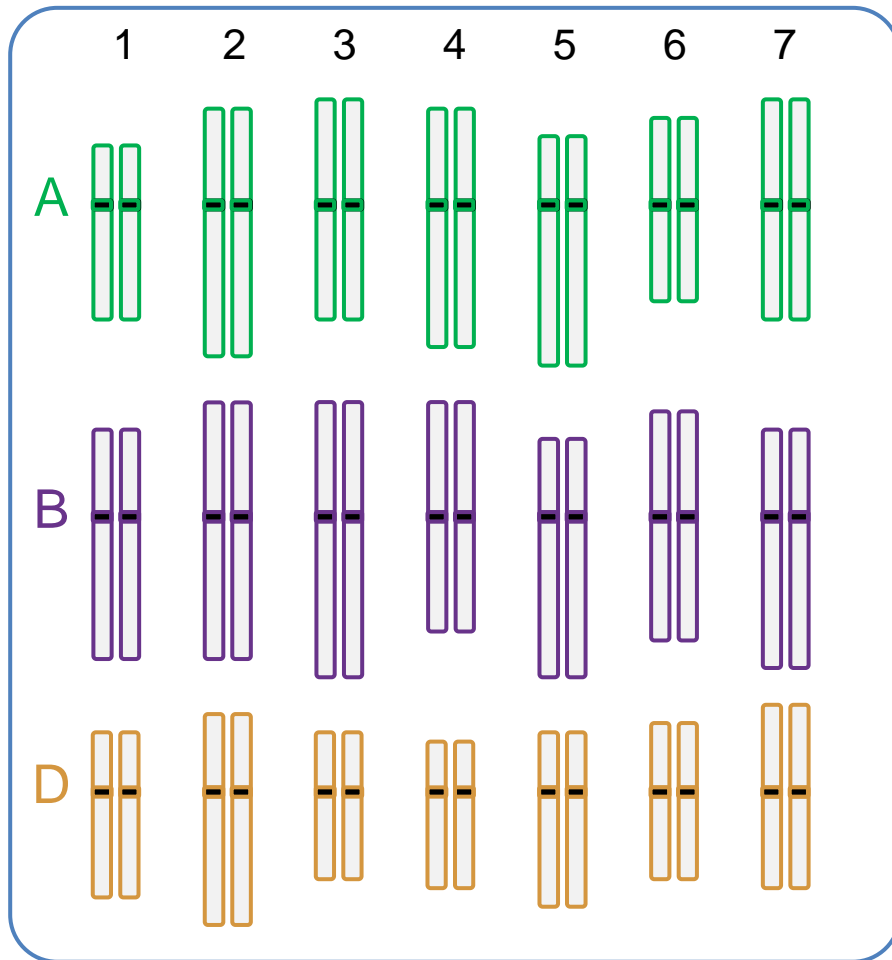


AABBDD *Triticum aestivum*

AABB *Triticum durum*

BB *Aegilops speltoides*





## Complex genome

- 15 Gb
- Hexaploid AA-BB-DD
- 85% TEs
- Ref cultivar: Chinese Spring



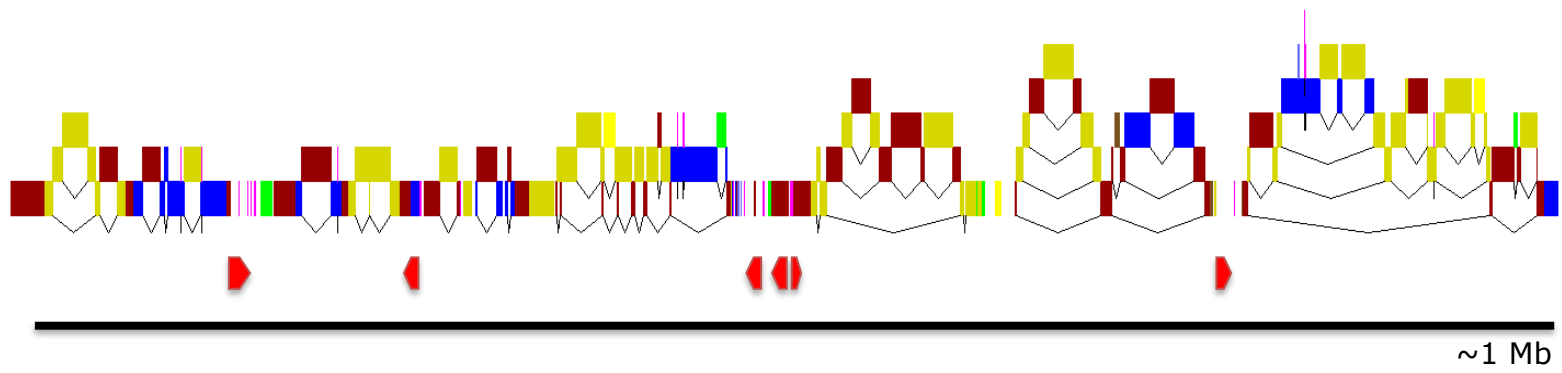
## Assembly (v1)

- 21 chromosomes (14.5 Gb)

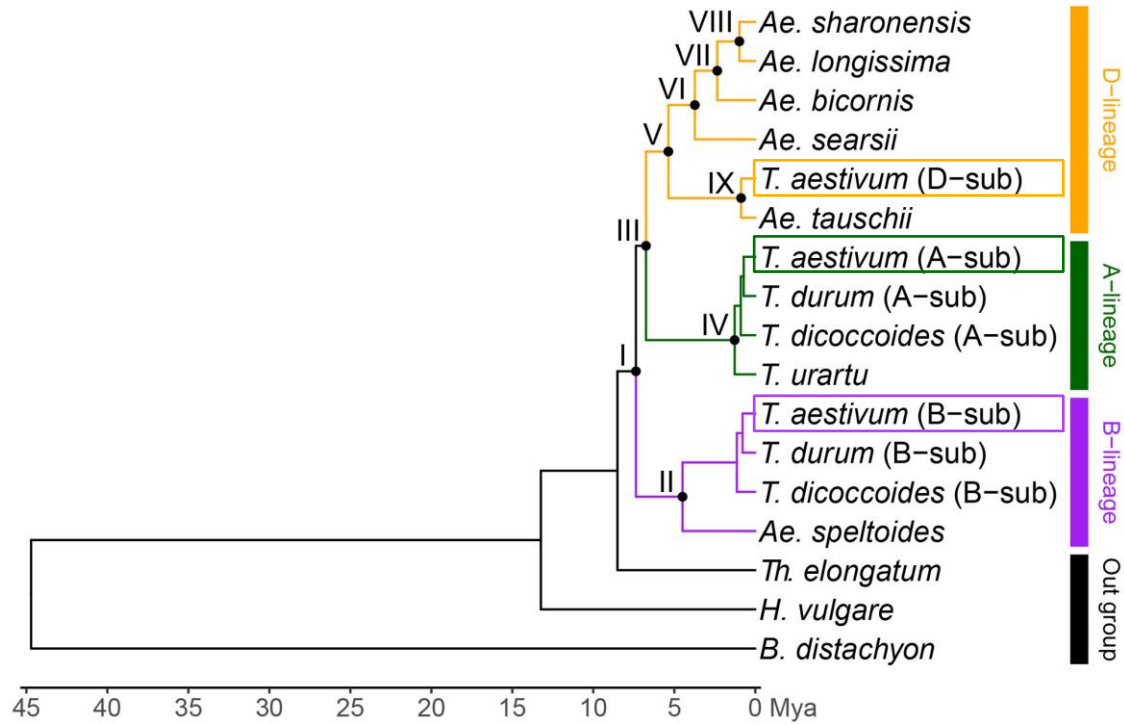
## Annotation (v1.1)

- 107k genes
- 4 million TEs





○ RQAs: **Reference-Quality Assemblies** of *Triticeae* genomes

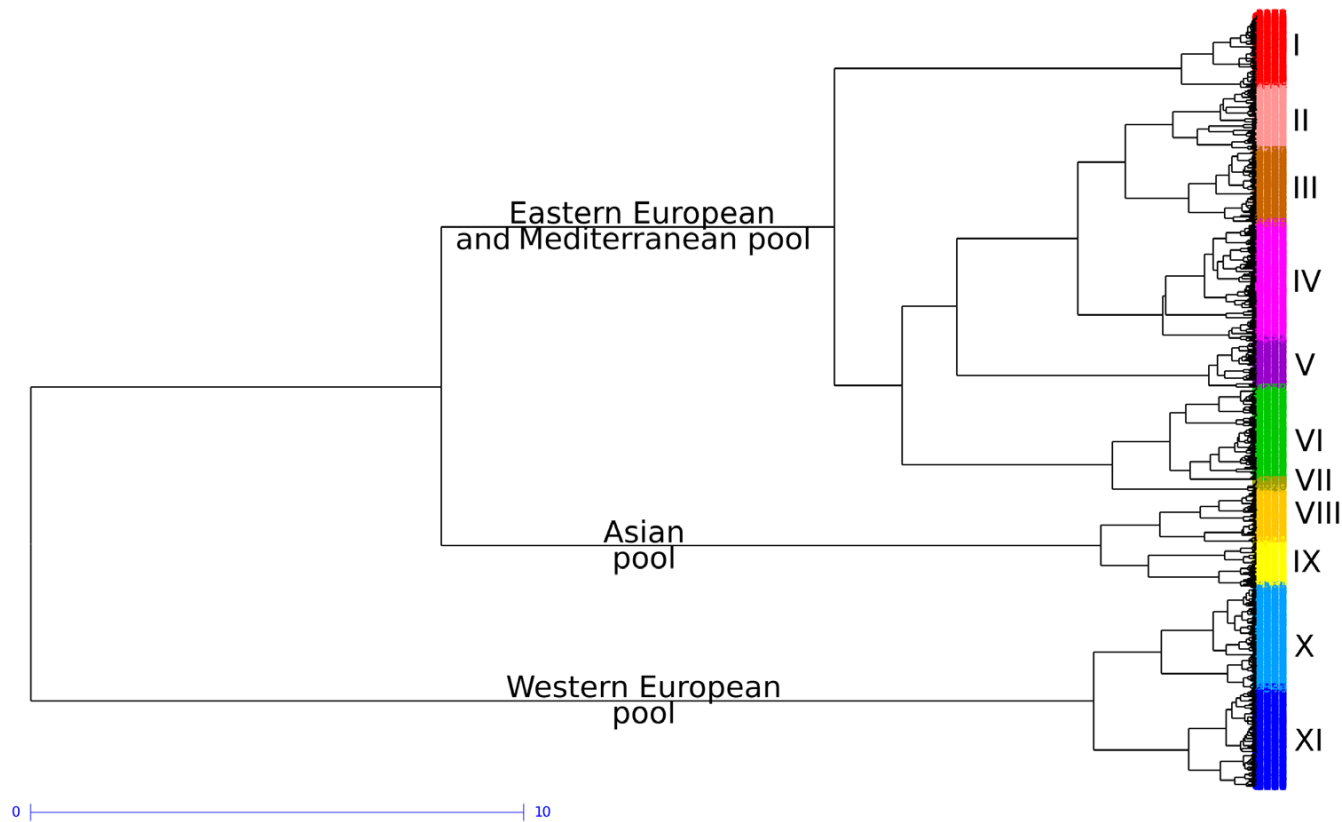


Li et al., Mol Plant 2022

➤ *T. aestivum*:  
Chinese Spring + **17** accessions

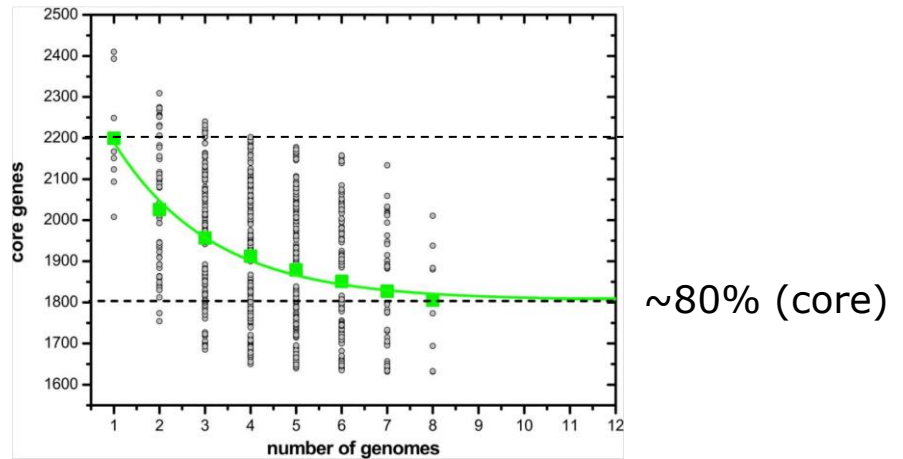
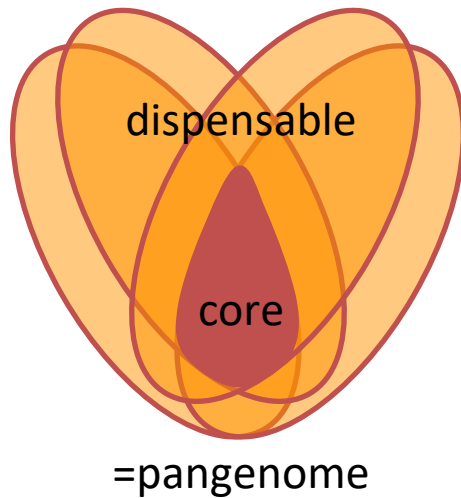
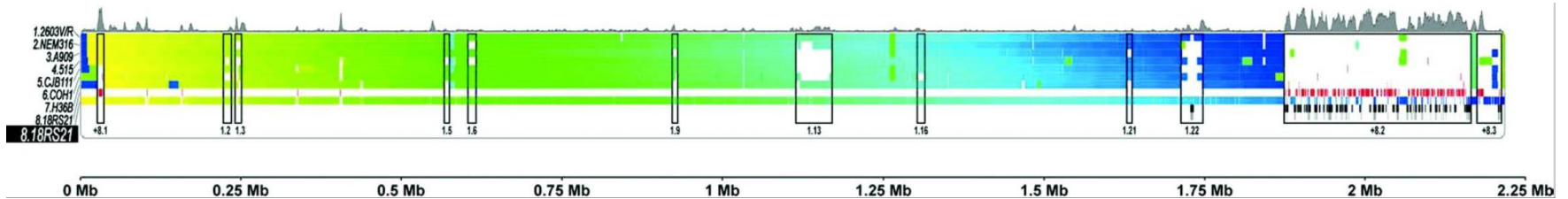


- **SNP** genotyping of **4500** accessions representing world-wide diversity

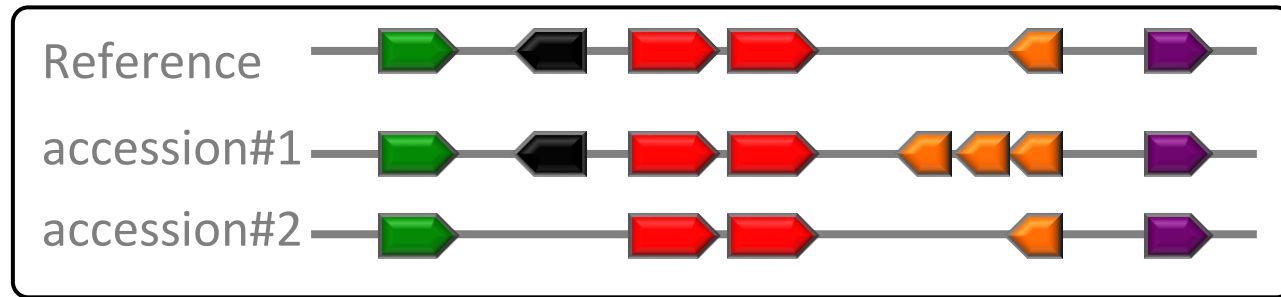


# ○ Pangenomics

➤ **Tettelin et al. PNAS 2005**  
8 genomes of *S. agalactiae*



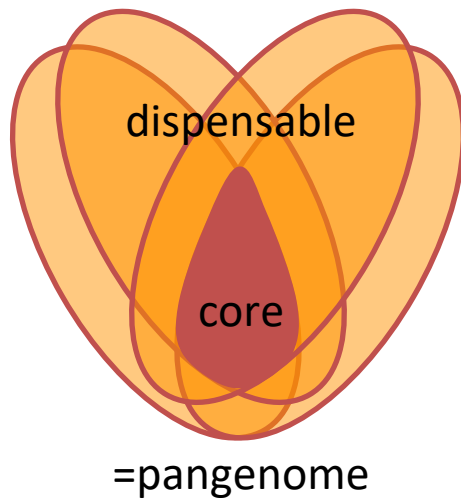
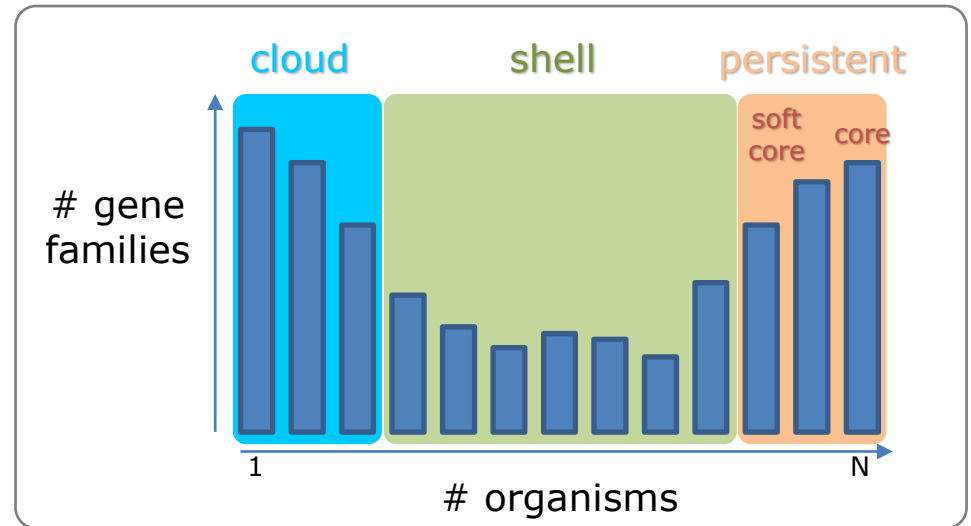
# ○ Structural Variations (SVs)



PAV

CNV

➤ **Koonin and Wolf NAR 2008**  
bacteria, archea (~700 genomes)



# ○ Pangenomics in plants

## *De novo* assembly of soybean wild relatives for pan-genome analysis of diversity and agronomic traits

Ying-hui Li<sup>1-3,11</sup>, Guangyu Zhou<sup>4,10,11</sup>, Jianxin Ma<sup>5,11</sup>, Wenkai Jiang<sup>4,11</sup>, Long-guo Jin<sup>1-3</sup>, Zhouhao Zhang<sup>4</sup>, Yong Guo<sup>1-3</sup>, Jinbo Zhang<sup>4</sup>, Yi Sui<sup>1-3</sup>, Liangtao Zheng<sup>4</sup>, Shan-shan Zhang<sup>1-3</sup>, Qiyang Zuo<sup>4</sup>, Xue-hui Shi<sup>1-3</sup>, Yan-fei Li<sup>1-3</sup>, Wan-ke Zhang<sup>6</sup>, Yiyao Hu<sup>4</sup>, Guanyi Kong<sup>4</sup>, Hui-long Hong<sup>1-3</sup>, Bing Tan<sup>1-3</sup>, Jian Song<sup>1-3</sup>, Zhang-xiong Liu<sup>1-3</sup>, Yaoshen Wang<sup>4</sup>, Hang Ruan<sup>4</sup>, Carol K L Yeung<sup>4</sup>, Jian Liu<sup>4</sup>, Hailong Wang<sup>4</sup>, Li-juan Zhang<sup>1-3</sup>, Rong-xia Guan<sup>1-3</sup>, Ke-jing Wang<sup>1-3</sup>, Wen-bin Li<sup>7</sup>, Shou-yi Chen<sup>6</sup>, Ru-zhen Chang<sup>1-3</sup>, Zhi Jiang<sup>4</sup>, Scott A Jackson<sup>8</sup>, Ruiqiang Li<sup>4,9</sup> & Li-juan Qiu<sup>1-3</sup>

NATURE BIOTECHNOLOGY | VOLUME 32 | NUMBER 10 | OCTOBER 2014

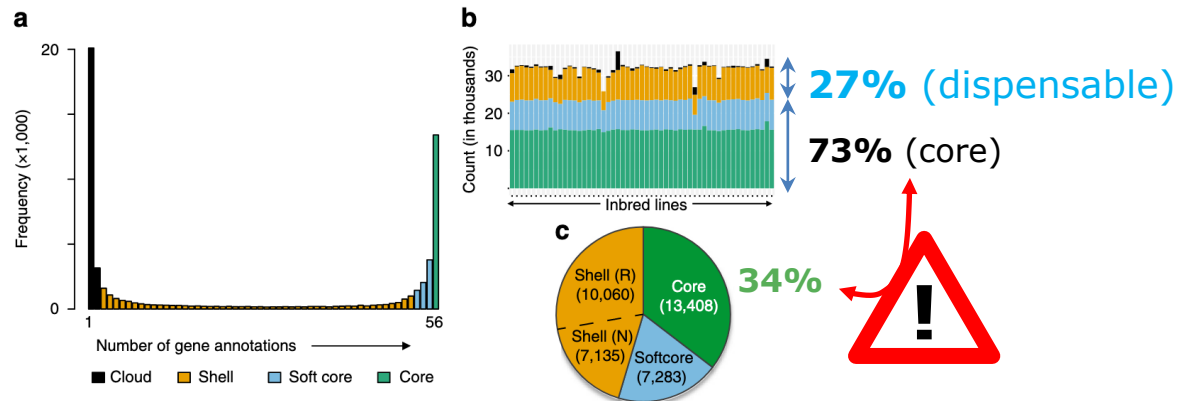
7 genomes of *Glycine soja* dispensable: ~20% genes

## Extensive gene content variation in the *Brachypodium distachyon* pan-genome correlates with population structure

Sean P. Gordon<sup>1</sup>, Bruno Contreras-Moreira<sup>2,3,4</sup>, Daniel P. Woods<sup>5,6</sup>, David L. Des Marais<sup>7,17</sup>, Diane Burgess<sup>8</sup>, Shengqiang Shu<sup>1</sup>, Christoph Stritt<sup>9</sup>, Anne C. Roulin<sup>9</sup>, Wendy Schackwitz<sup>1</sup>, Ludmila Tyler<sup>10</sup>, Joel Martin<sup>1</sup>, Anna Lipzen<sup>1</sup>, Niklas Dochy<sup>11</sup>, Jeremy Phillips<sup>1</sup>, Kerrie Barry<sup>1</sup>, Koen Geuten<sup>11</sup>, Hikmet Budak<sup>12</sup>, Thomas E. Juenger<sup>13</sup>, Richard Amasino<sup>5,6</sup>, Ana L. Caicedo<sup>10</sup>, David Goodstein<sup>1</sup>, Patrick Davidson<sup>1</sup>, Luis A. J. Mur<sup>14</sup>, Melania Figueroa<sup>15</sup>, Michael Freeling<sup>8</sup>, Pilar Catalan<sup>4,16</sup> & John P. Vogel<sup>1,8</sup>

NATURE COMMUNICATIONS | 8: 2184 | DOI: 10.1038/s41467-017-02292-8 | www.nature.com/naturecommunications | 2017

## 54 genomes of *Brachypodium distachyon*



# ○ Pangenomics in plants

## The pangenome of an agronomically important crop plant *Brassica oleracea*

Agnieszka A. Golicz<sup>1</sup>, Philipp E. Bayer<sup>2</sup>, Guy C. Barker<sup>3</sup>, Patrick P. Edger<sup>4</sup>, HyeRan Kim<sup>5</sup>, Paula A. Martinez<sup>1</sup>, Chon Kit Kenneth Chan<sup>2</sup>, Anita Severn-Ellis<sup>2</sup>, W. Richard McCombie<sup>6</sup>, Isobel A.P. Parkin<sup>7</sup>, Andrew H. Paterson<sup>8</sup>, J. Chris Pires<sup>9</sup>, Andrew G. Sharpe<sup>10</sup>, Haibao Tang<sup>11</sup>, Graham R. Teakle<sup>3</sup>, Christopher D. Town<sup>12</sup>, Jacqueline Batley<sup>2</sup> & David Edwards<sup>2</sup>

NATURE COMMUNICATIONS | 7:13390 | DOI: 10.1038/ncomms13390 | www.nature.com/naturecommunications 2016

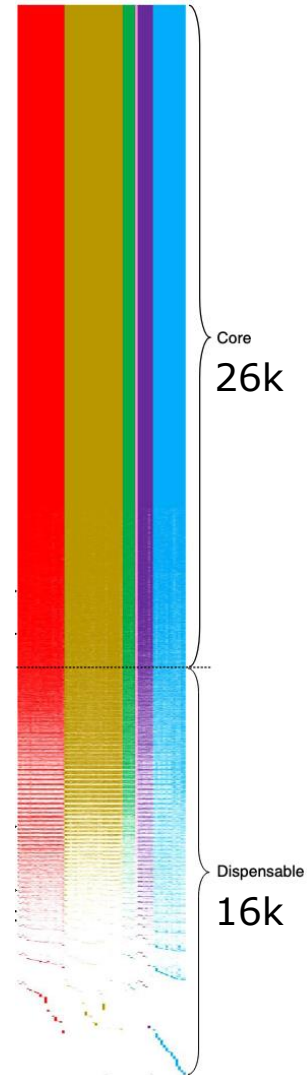
9 genomes of *Brassica oleracea*  
dispensable: ~20% genes

## Pan-genome analysis highlights the extent of genomic variation in cultivated and wild rice

Qiang Zhao<sup>1</sup>, Qi Feng<sup>1</sup>, Hengyun Lu<sup>1</sup>, Yan Li<sup>1</sup>, Ahong Wang<sup>1</sup>, Qilin Tian<sup>1</sup>, Qilin Zhan<sup>1</sup>, Yiqi Lu<sup>1</sup>, Lei Zhang<sup>1</sup>, Tao Huang<sup>1</sup>, Yongchun Wang<sup>1</sup>, Danlin Fan<sup>1</sup>, Yan Zhao<sup>1</sup>, Ziqun Wang<sup>1</sup>, Congcong Zhou<sup>1</sup>, Jiaying Chen<sup>1</sup>, Chuanrang Zhu<sup>1</sup>, Wenjun Li<sup>1</sup>, Qijun Weng<sup>1</sup>, Qun Xu<sup>2</sup>, Zi-Xuan Wang<sup>1</sup>, Xinghua Wei<sup>2</sup>, Bin Han<sup>1</sup> and Xuehui Huang<sup>1,3\*</sup>

NATURE GENETICS | VOL 50 | FEBRUARY 2018 | 278-284 | www.nature.com/naturegenetics

67 genomes of *Oryza sativa* + *rufipogon*  
~20% genes



# ○ Pangenomics in plants

The Plant Cell, Vol. 26: 121–135, January 2014, www.plantcell.org © 2014.

## Insights into the Maize Pan-Genome and Pan-Transcriptome <sup>WIOOPEN</sup>


Candice N. Hirsch,<sup>a,b,1</sup> Jillian M. Foerster,<sup>c,2</sup> James M. Johnson,<sup>c,3</sup> Rajandeep S. Sekhon,<sup>c,d</sup> German Muttoni,<sup>c,4</sup> Brienne Vaillancourt,<sup>a,b</sup> Francisco Peñagaricano,<sup>o</sup> Erika Lindquist,<sup>f</sup> Mary Ann Pedraza,<sup>f</sup> Kerrie Barry,<sup>f</sup> Natalia de Leon,<sup>c,d</sup> Shawn M. Kaeppler,<sup>c,d</sup> and C. Robin Buell<sup>a,b,5</sup>

503 **maize** accessions (RNASeq)  
=> ~8700 novel transcripts

Darracq *et al.* *BMC Genomics* (2018) 19:119  
DOI 10.1186/s12864-018-4490-7

## Sequence analysis of European maize inbred line F2 provides new insights into molecular and chromosomal characteristics of presence/absence variants



Aude Darracq<sup>1†</sup>, Clémentine Vitte<sup>1†</sup>, Stéphane Nicolas<sup>1</sup>, Jorge Duarte<sup>2</sup>, Jean-Philippe Pichon<sup>2</sup>, Tristan Mary-Huard<sup>1,3</sup>, Céline Chevalier<sup>1</sup>, Aurélie Bérard<sup>4</sup>, Marie-Christine Le Paslier<sup>4</sup>, Peter Rogowsky<sup>5</sup>, Alain Charcosset<sup>1</sup> and Johann Joets<sup>1\*</sup> 

### Dispensable vs core genes:

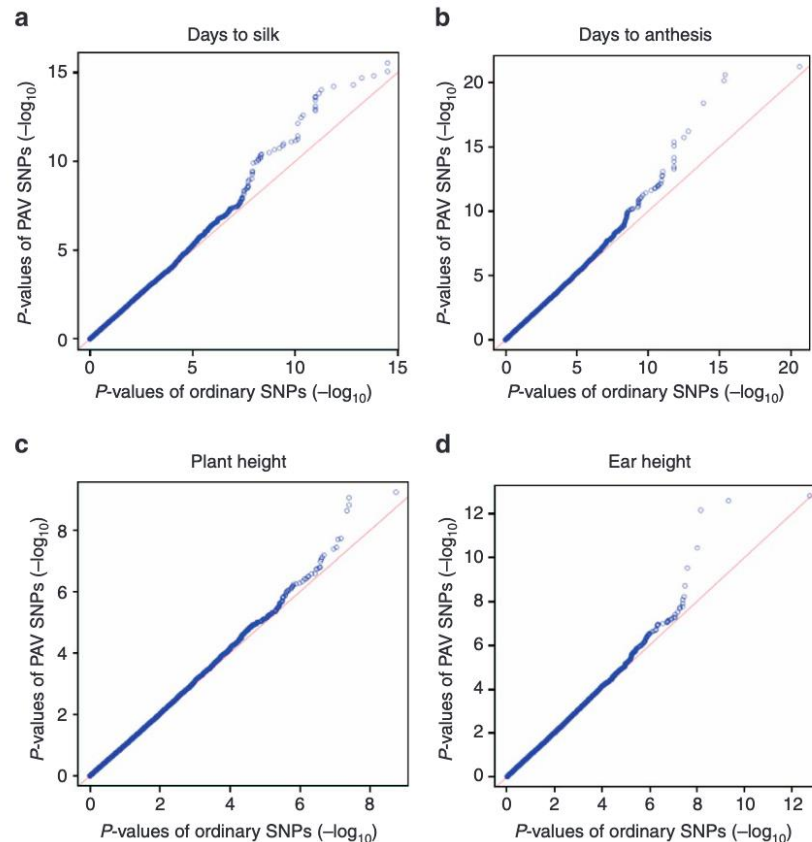
- **shorter**, fewer introns
- less **expressed**, fewer conditions
- **evolve** more rapidly
- enriched in **functions** related to adaptation, response to env

# ○ Pangenomics in plants

## High-resolution genetic mapping of maize pan-genome sequence anchors

Fei Lu<sup>1</sup>, Maria C. Romay<sup>1</sup>, Jeffrey C. Glaubitz<sup>1</sup>, Peter J. Bradbury<sup>2</sup>, Robert J. Elshire<sup>1</sup>, Tianyu Wang<sup>3</sup>, Yu Li<sup>3</sup>, Yongxiang Li<sup>3</sup>, Kassa Semagn<sup>4</sup>, Xuecai Zhang<sup>5</sup>, Alvaro G. Hernandez<sup>6</sup>, Mark A. Mikel<sup>6,7</sup>, Ilya Soifer<sup>8</sup>, Omer Barad<sup>8</sup> & Edward S. Buckler<sup>1,2</sup>

NATURE COMMUNICATIONS | 6:6914 | DOI: 10.1038/ncomms7914 | www.nature.com/naturecommunications 2015



=> "PAV SNPs exhibit enriched associations with traits"



# ○ Pangenomics in plants

## The tomato pan-genome uncovers new genes and a rare allele regulating fruit flavor

Lei Gao<sup>1,10</sup>, Itay Gonda<sup>1,2,10</sup>, Honghe Sun<sup>1</sup>, Qiyue Ma<sup>1</sup>, Kan Bao<sup>1</sup>, Denise M. Tieman<sup>3</sup>, Elizabeth A. Burzynski-Chang<sup>4</sup>, Tara L. Fish<sup>5</sup>, Kaitlin A. Stromberg<sup>1</sup>, Gavin L. Sacks<sup>4</sup>, Theodore W. Thannhauser<sup>5</sup>, Majid R. Foolad<sup>6</sup>, Maria Jose Diez<sup>7</sup>, Jose Blanca<sup>7</sup>, Joaquin Canizares<sup>7</sup>, Yimin Xu<sup>1</sup>, Esther van der Knaap<sup>8</sup>, Sanwen Huang<sup>9</sup>, Harry J. Klee<sup>3</sup>, James J. Giovannoni<sup>1,5\*</sup> and Zhangjun Fei<sup>1,5\*</sup>

NATURE GENETICS | VOL 51 | JUNE 2019 | 1044-1051 | [www.nature.com/naturegenetics](http://www.nature.com/naturegenetics)

725 genomes of *Solanum* cultivated + 3 wild species  
core: **74%** genes

"The most striking feature of the tomato pan-genome was its **high core** gene content (74.2%), as compared with those of *Arabidopsis thaliana* (70%), *Brassica napus* (62%), *bread wheat* (64%), *rice* (54%), *wild soybean* (49%) and *Brachypodium distachyon* (35%)"

# ○ Pangenomics in plants

## The pangenome of hexaploid bread wheat

Juan D. Montenegro<sup>1,†</sup>, Agnieszka A. Golicz<sup>1,2,†,‡</sup>, Philipp E. Bayer<sup>2,†</sup>, Bhavna Hurgobin<sup>1,2</sup>, HueyTyng Lee<sup>1,2</sup>, Chon-Kit Kenneth Chan<sup>2</sup>, Paul Visendi<sup>1</sup>, Kaitao Lai<sup>3</sup>, Jaroslav Doležel<sup>4</sup>, Jacqueline Batley<sup>1,2,5</sup> and David Edwards<sup>1,2,5,\*</sup>

*The Plant Journal* (2017) **90**, 1007–1013

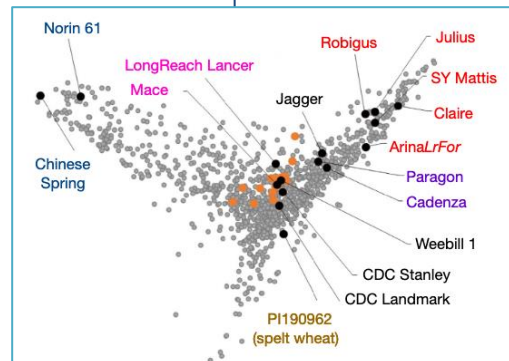
16 Australian wheat cultivars (Illumina reseq)  
dispensable: ~35% of genes

15 wheat genomes assembled

- "12% of genes showed PAVs"
- "26% of the projected genes were found in tandem duplications, indicating that CNV is a strong contributor of genetic variation in wheat"

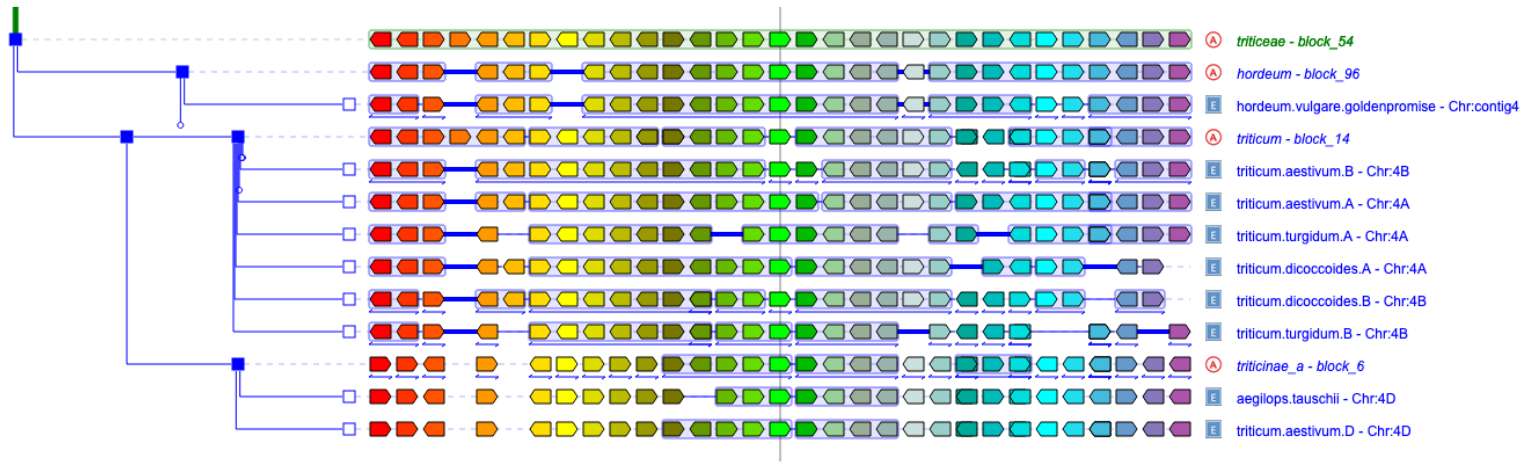
## Multiple wheat genomes reveal global variation in modern breeding

<https://doi.org/10.1038/s41586-020-2961-x>



Sean Walkowiak<sup>1,2,4,†</sup>, Liangliang Gao<sup>3,4,†</sup>, Cecile Monat<sup>4,4,†</sup>, Georg Haberer<sup>5</sup>, Muluaem T. Kassa<sup>6</sup>, Jemima Brinton<sup>7</sup>, Ricardo H. Ramirez-Gonzalez<sup>7</sup>, Markus C. Kolodziej<sup>8</sup>, Emily Delorean<sup>9</sup>, Dinushika Thambugala<sup>9</sup>, Valentyna Klymiuk<sup>1</sup>, Brook Byrns<sup>1</sup>, Heidrun Gundlach<sup>6</sup>, Venkat Bandi<sup>10</sup>, Jorge Nunez Siri<sup>10</sup>, Kirby Nilsen<sup>11</sup>, Catharine Aquino<sup>12</sup>, Axel Himmelbach<sup>4</sup>, Dario Copetti<sup>13,14</sup>, Tomohiro Ban<sup>15</sup>, Luca Venturini<sup>16</sup>, Michael Bevan<sup>7</sup>, Bernardo Clavijo<sup>17</sup>, Dal-Hoe Koo<sup>7</sup>, Jennifer Ens<sup>1</sup>, Krystalee Wiebe<sup>1</sup>, Amidou N'Diaye<sup>1</sup>, Allen K. Fritz<sup>2</sup>, Carl Gutwin<sup>10</sup>, Anne Fiebig<sup>4</sup>, Christine Fosker<sup>17</sup>, Bin Xiao Fu<sup>2</sup>, Gonzalo Garcia Accinelli<sup>17</sup>, Keith A. Gardner<sup>18</sup>, Nick Fradgley<sup>18</sup>, Juan Gutierrez-Gonzalez<sup>19</sup>, Gwyneth Halstead-Nussloch<sup>12</sup>, Masaomi Hatakeyama<sup>22,23</sup>, Chu Shin Koh<sup>20</sup>, Jasline Deek<sup>21</sup>, Alejandro C. Costamagna<sup>22</sup>, Pierre Fobert<sup>2</sup>, Darren Heavens<sup>17</sup>, Hiroyuki Kanamori<sup>23</sup>, Kanako Kawaura<sup>24</sup>, Fuminori Kobayashi<sup>23</sup>, Ksenia Krasileva<sup>25</sup>, Tony Kuo<sup>24,25</sup>, Neil McKenzie<sup>7</sup>, Kazuki Murata<sup>26</sup>, Yusuke Nabeka<sup>26</sup>, Timothy Paape<sup>13</sup>, Sudharsan Padmarasu<sup>4</sup>, Lawrence Percival-Alwyn<sup>19</sup>, Sateesh Kagale<sup>6</sup>, Uwe Scholz<sup>2</sup>, Jun Sese<sup>26,27</sup>, Philomin Juliana<sup>28</sup>, Ravi Singh<sup>28</sup>, Rie Shimizu-Inatsugi<sup>13</sup>, David Swarbrick<sup>17</sup>, James Cockram<sup>18</sup>, Hikmet Budak<sup>29</sup>, Toshiaki Tameshige<sup>19</sup>, Tsuyoshi Tanaka<sup>23</sup>, Hiroyuki Tsujii<sup>15</sup>, Jonathan Wright<sup>17</sup>, Jianzhong Wu<sup>23</sup>, Burkhard Steuernagel<sup>7</sup>, Ian Small<sup>30</sup>, Sylvie Cloutier<sup>31</sup>, Gabriel Keeble-Gagnère<sup>32</sup>, Gary Muehlbauer<sup>19</sup>, Josquin Tibbets<sup>32</sup>, Shuhei Nasuda<sup>30</sup>, Joanna Melonek<sup>30</sup>, Pierre J. Hucl<sup>1</sup>, Andrew G. Sharpe<sup>30</sup>, Matthew Clark<sup>16</sup>, Erik Legg<sup>33</sup>, Arvind Bharti<sup>33</sup>, Peter Langridge<sup>34</sup>, Anthony Hall<sup>17</sup>, Cristobal Uauy<sup>7</sup>, Martin Mascher<sup>4,35</sup>, Simon G. Krattinger<sup>8,36</sup>, Hirokazu Handa<sup>23,37</sup>, Kentaro K. Shimizu<sup>13,15</sup>, Assaf Distelfeld<sup>38</sup>, Ken Chalmers<sup>34</sup>, Beat Keller<sup>8</sup>, Klaus F. X. Mayer<sup>5,39</sup>, Jesse Poland<sup>9</sup>, Nils Stein<sup>4,40</sup>, Curt A. McCartney<sup>9,41</sup>, Manuel Spannagl<sup>5,42</sup>, Thomas Wicker<sup>5,42</sup> & Curtis J. Pozniak<sup>1,43</sup>

# ○ Build and visualize pangeneromes

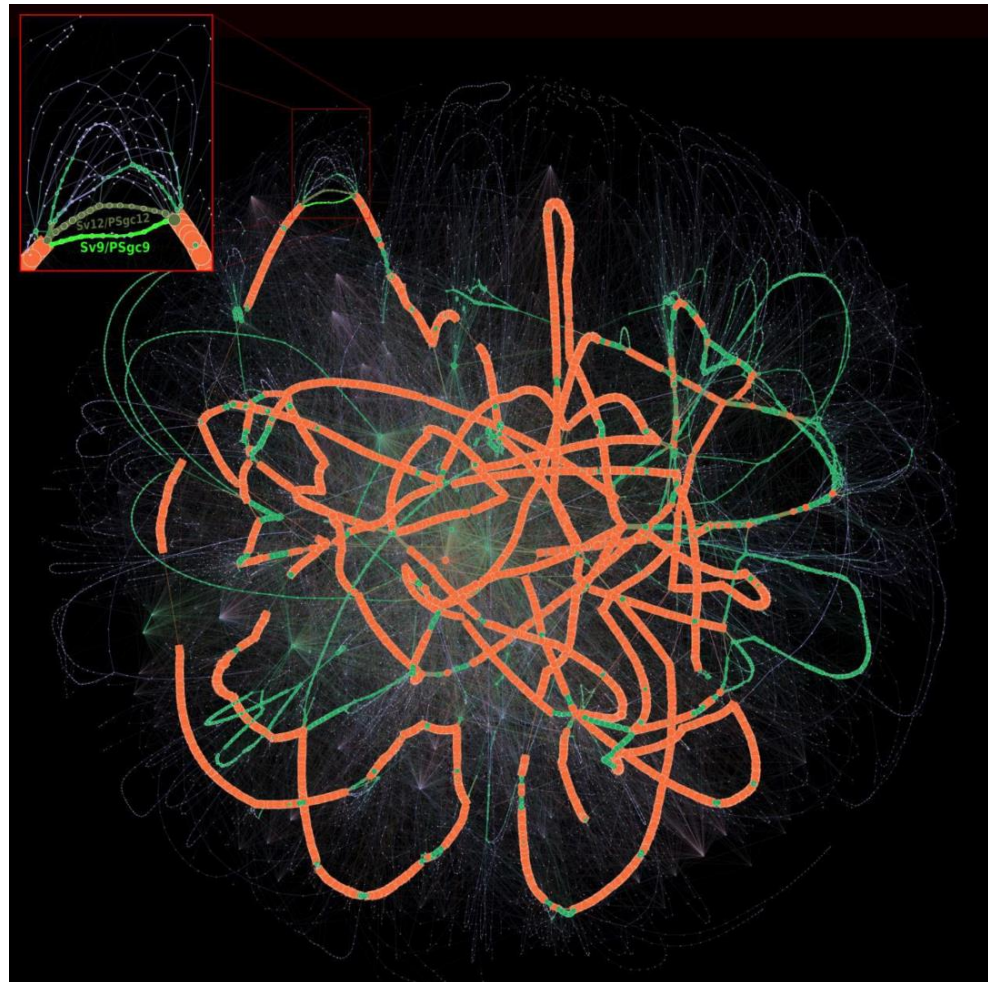


GenomicusPlants — database version: 49.01 / Web-code version: 2021-08-15 — Dyogen Team

➤ **Gautereau et al. Plos Comput. Biol. 2020**

*pangenome graph of  
~3000 Acinetobacter*

PPanGGOLiN



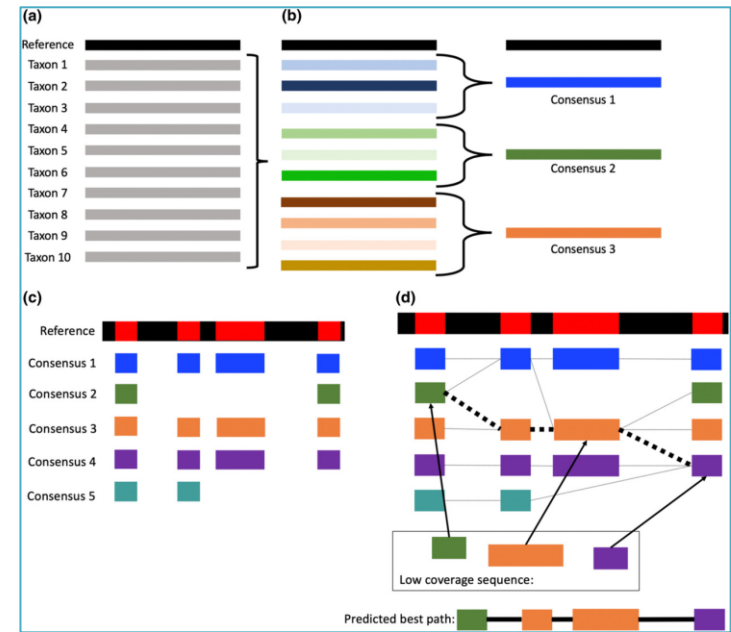
# ○ Practical Haplotype Graph

## A sorghum practical haplotype graph facilitates genome-wide imputation and cost-effective genomic prediction

Sarah E. Jensen<sup>1</sup> | Jean Rigaud Charles<sup>2</sup> | Kebede Muleta<sup>3</sup> |  
 Peter J. Bradbury<sup>4,6</sup> | Terry Casstevens<sup>4</sup> | Santosh P. Deshpande<sup>5</sup> |  
 Michael A. Gore<sup>1</sup> | Rajeev Gupta<sup>5</sup> | Daniel C. Ilut<sup>1</sup> | Lynn Johnson<sup>4</sup> |  
 Roberto Lozano<sup>1</sup> | Zachary Miller<sup>4</sup> | Punna Ramu<sup>4</sup> | Abhishek Rathore<sup>5</sup> |  
 M. Cinta Romay<sup>4</sup> | Hari D. Upadhyaya<sup>5</sup> | Rajeev K. Varshney<sup>5</sup> |  
 Geoffrey P. Morris<sup>3</sup> | Gael Pressoir<sup>2</sup> | Edward S. Buckler<sup>1,4,6</sup> |  
 Guillaume P. Ramstein<sup>4</sup>

*Plant Genome*. 2020;13:e20009.  
<https://doi.org/10.1002/tpg2.20009>

- for breeding programs
- based on a limited number of founder lines
- **0.01x** skim-seq -> call SNPs with 5% errors



## Development of the Wheat Practical Haplotype Graph database as a resource for genotyping data storage and genotype imputation

Katherine W. Jordan<sup>1,2,†</sup> | Peter J. Bradbury<sup>3</sup> | Zachary R. Miller<sup>4</sup> | Moses Nyine<sup>1</sup> | Fei He<sup>1</sup> | Max Fraser<sup>5</sup> | Jim Anderson<sup>5</sup> | Esten Mason<sup>6</sup> | Andrew Katz<sup>6</sup> | Stephen Pearce<sup>6</sup> | Arron H. Carter<sup>7</sup> | Samuel Prather<sup>7</sup> | Michael Pumphrey<sup>7</sup> | Jianli Chen<sup>8</sup> | Jason Cook<sup>9</sup> | Shuyu Liu<sup>10</sup> | Jackie C. Rudd<sup>10</sup> | Zhen Wang<sup>10</sup> | Chenggen Chu<sup>10</sup> | Amir M. H. Ibrahim<sup>10</sup> | Jonathan Turkus<sup>11</sup> | Eric Olson<sup>11</sup> | Ragupathi Nagarajan<sup>12</sup> | Brett Carver<sup>12</sup> | Liuling Yan<sup>12</sup> | Ellie Taagen<sup>4</sup> | Mark Sorrells<sup>4</sup> | Brian Ward<sup>13</sup> | Jie Ren<sup>1,14</sup> | Alina Akhunova<sup>1,14</sup> | Guihua Bai<sup>2</sup> | Robert Bowden<sup>2</sup> | Jason Fiedler<sup>15</sup> | Justin Faris<sup>15</sup> | Jorge Dubcovsky<sup>16</sup> | Mary Guttieri<sup>2</sup> | Gina Brown-Guedira<sup>13</sup> | Ed Buckler<sup>3</sup> | Jean-Luc Jannink<sup>3</sup> | and Eduard D. Akhunov<sup>1,\*</sup>

G3, 2022, 12(2), jkab390

- 65 wheat lines, exome capture
- 0.01x -> imputations with 8% errors

## 2. Wheat genome sequencing, annotation, A-B-D comparative genomics

**Shifting the limits in wheat research  
and breeding using a fully annotated  
reference genome**

**International Wheat Genome Sequencing Consortium (IWGSC)\***

*Science* **361**, 661 (2018) 17 August 2018

# ○ Predicting genes is still NOT routine

**GDEC** Clermont, France – *Rimbert Leroy Choulet et al.*  
**PGSB** Munich, Germany – *Spannagl Twardziok et al.*  
**EI** Norwich, UK – *Swarbreck Venturini et al.*

IWGSC RefSeq (Chinese Spring)

- 107,891 genes

**HC genes**

35345

35643

34212

107k

**LC genes**

51585

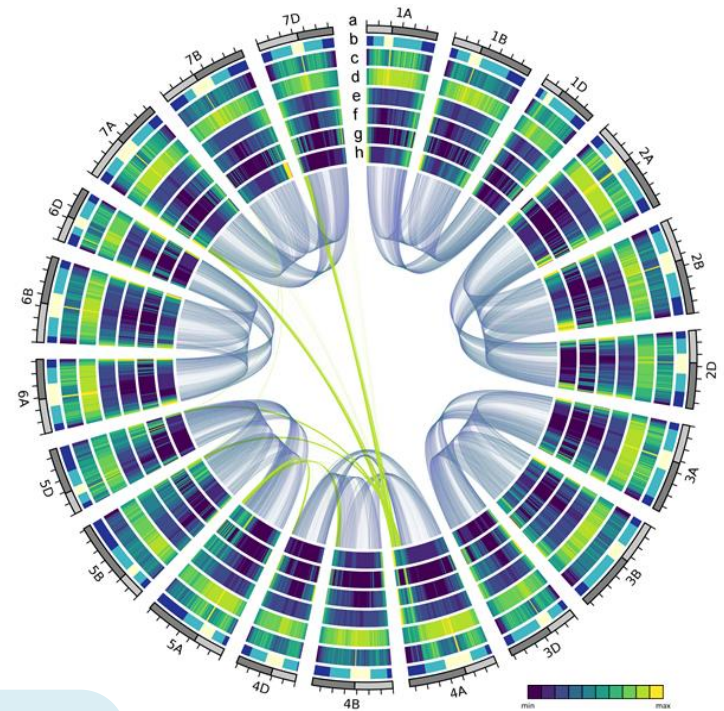
58359

44835

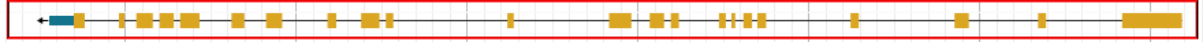
161k

**pseudogenes**

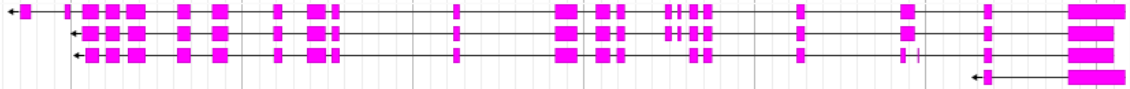
~300k



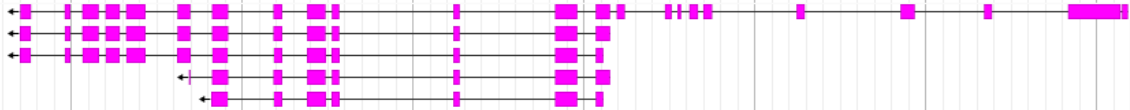
⊗ HighConfidenceGenesv1.1 (GOMAP annotation available)



⊗ Sorghum bicolor



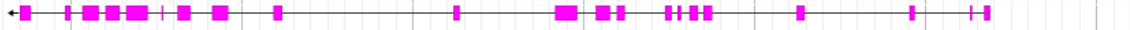
⊗ Hordeum vulgare var. distichum



⊗ Aegilops tauschii



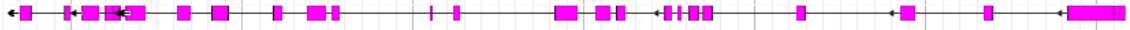
⊗ Triticum urartu



⊗ Brachypodium distachyon



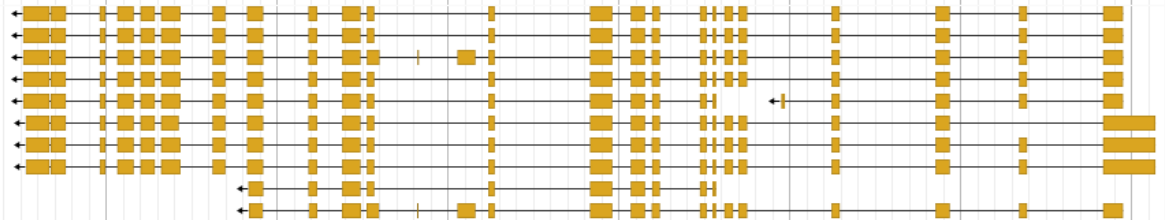
⊗ Oryza barthii



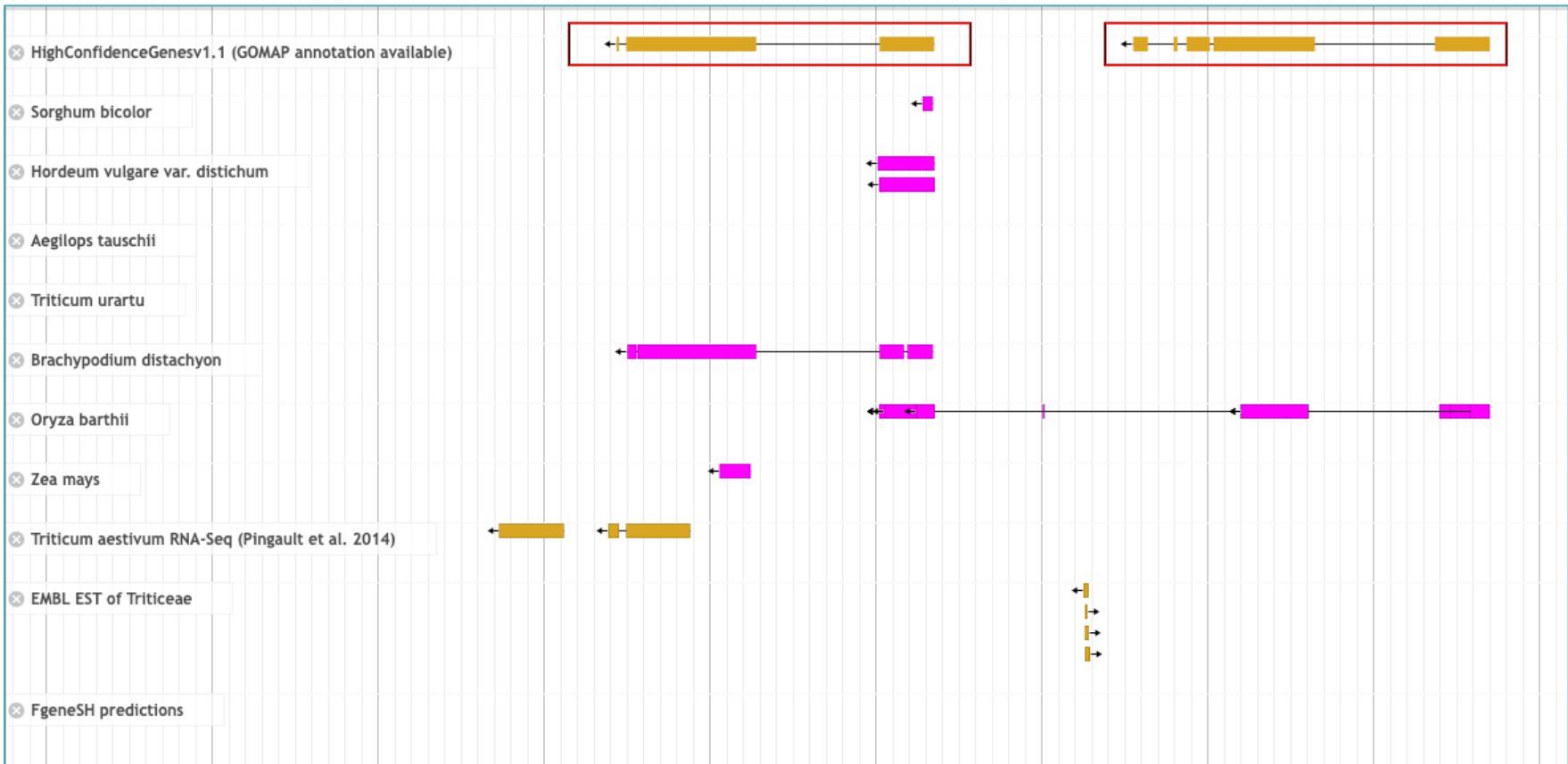
⊗ Zea mays



⊗ Triticum aestivum RNA-Seq (Pingault et al. 2014)



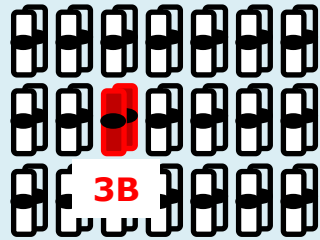




- Impact of annotation methods on knowledge

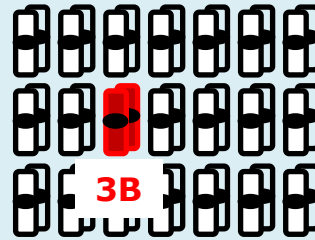
- in 2017: 3 versions of Chinese Spring chr3B

chr3B BAC-by-BAC



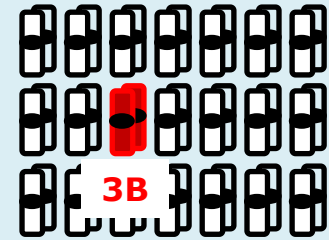
*Choulet et al. Science 2014*

TGAC\_v1



*Clavijo et al. Genom Biol 2017*

Chr. Survey Seq



*IWGSC Science 2014*

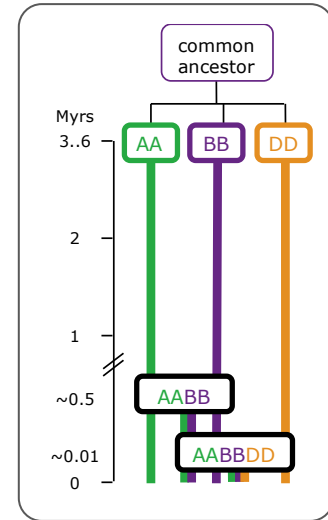
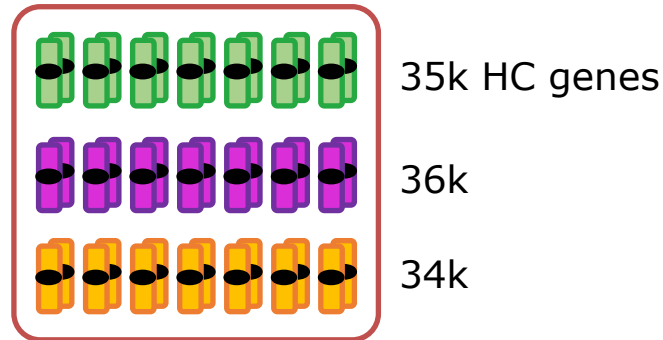
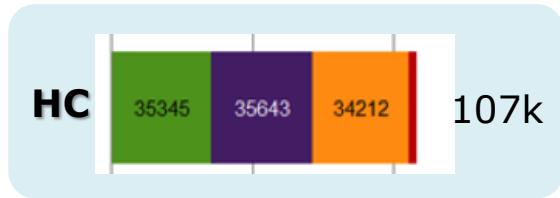
7264 genes

-> (26% identical)

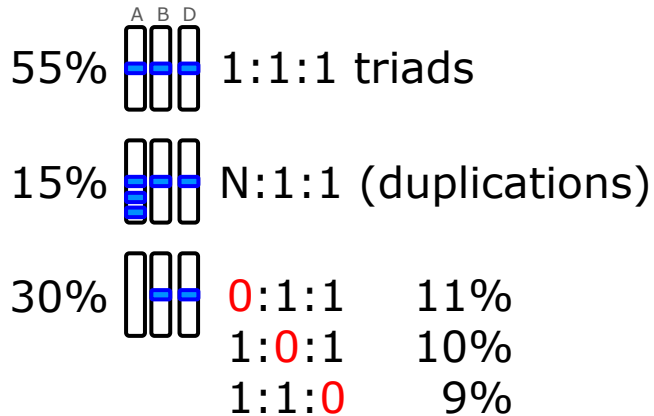
5728 genes

5862 genes  
(12% identical)

# ○ A-B-D Comparative Genomics

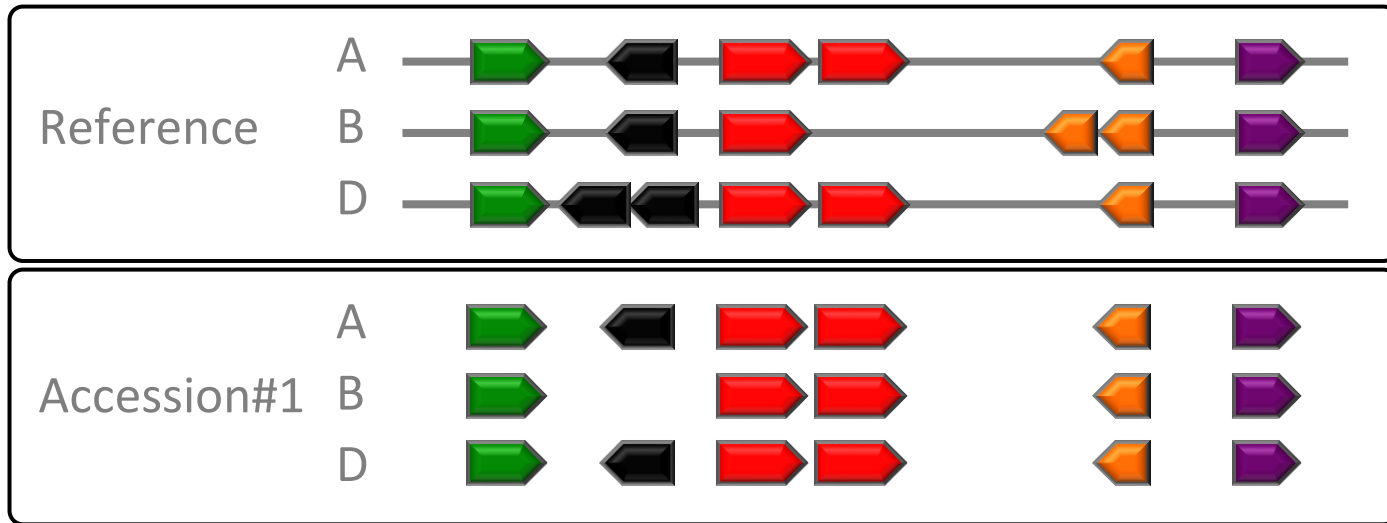


➤ 39k homeologous groups [ including **29k** LC genes ]



[ 27% of genes are duplicates ]  
ex: 207 PPRs

- Polyploidy + single gene duplications



# 3. A-B-D Comparative Analysis of **TEs**

Wicker *et al. Genome Biology* (2018) 19:103  
<https://doi.org/10.1186/s13059-018-1479-0>

Genome Biology

RESEARCH

Open Access

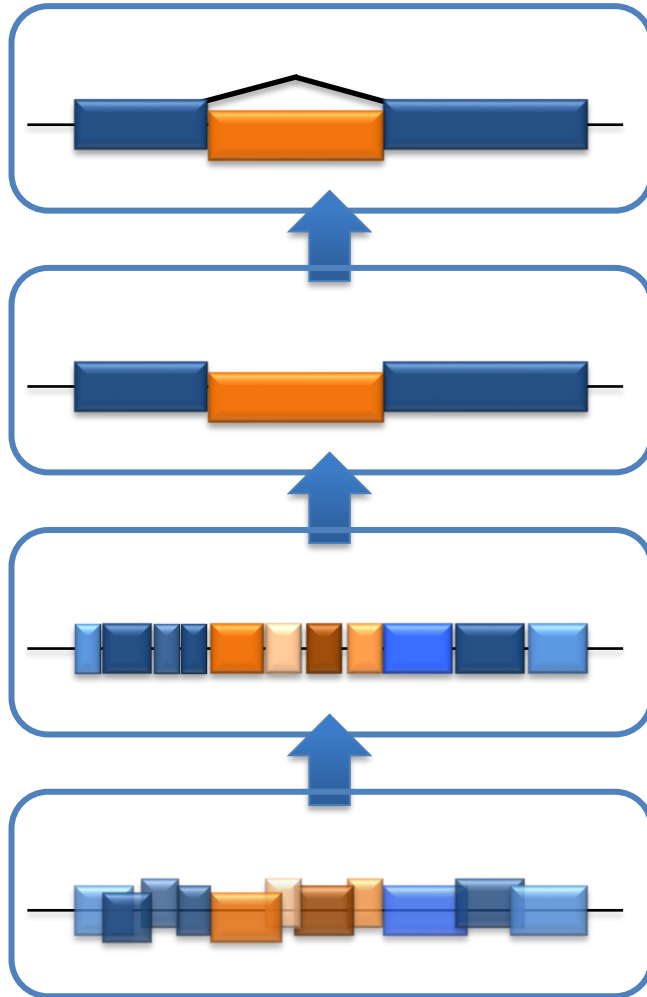


## Impact of transposable elements on genome structure and evolution in bread wheat

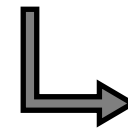
Thomas Wicker<sup>1†</sup>, Heidrun Gundlach<sup>2†</sup>, Manuel Spannagl<sup>2</sup>, Cristobal Uauy<sup>3</sup>, Philippa Borrill<sup>3</sup>, Ricardo H. Ramírez-González<sup>3</sup>, Romain De Oliveira<sup>4</sup>, International Wheat Genome Sequencing Consortium<sup>5</sup>, Klaus F. X. Mayer<sup>2,6</sup>, Etienne Paux<sup>4</sup> and Frédéric Choulet<sup>4\*</sup> 

# ○ TE modeling with **CLARI-TE** and **ClariTeRep**

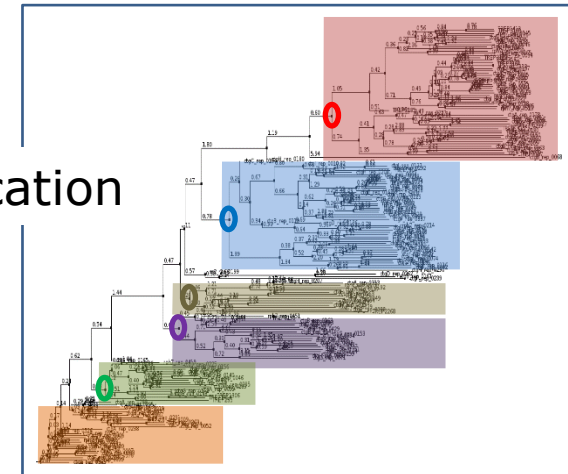
<https://github.com/jdaron/CLARI-TE>



~5000 known wheat TEs



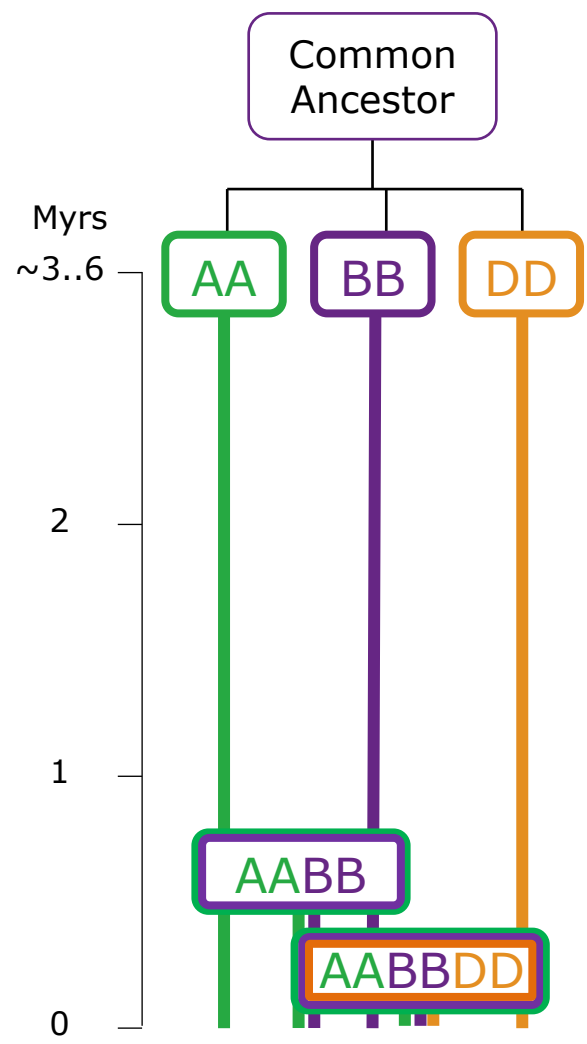
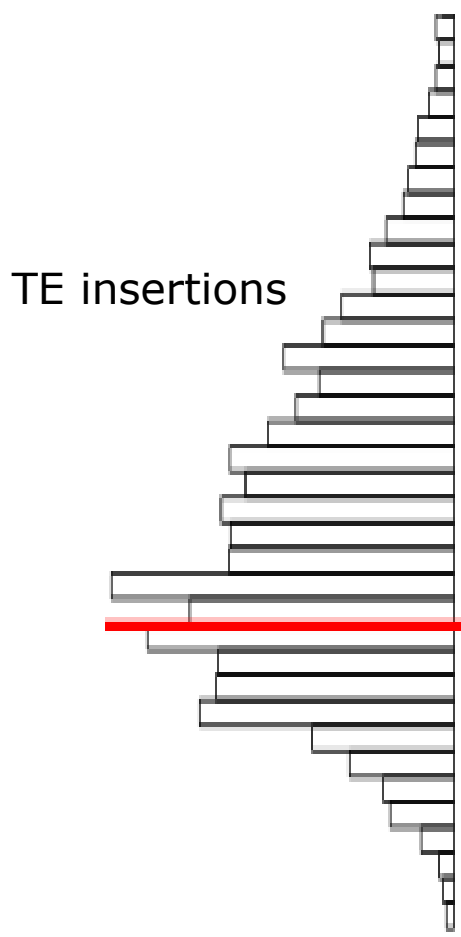
Classification

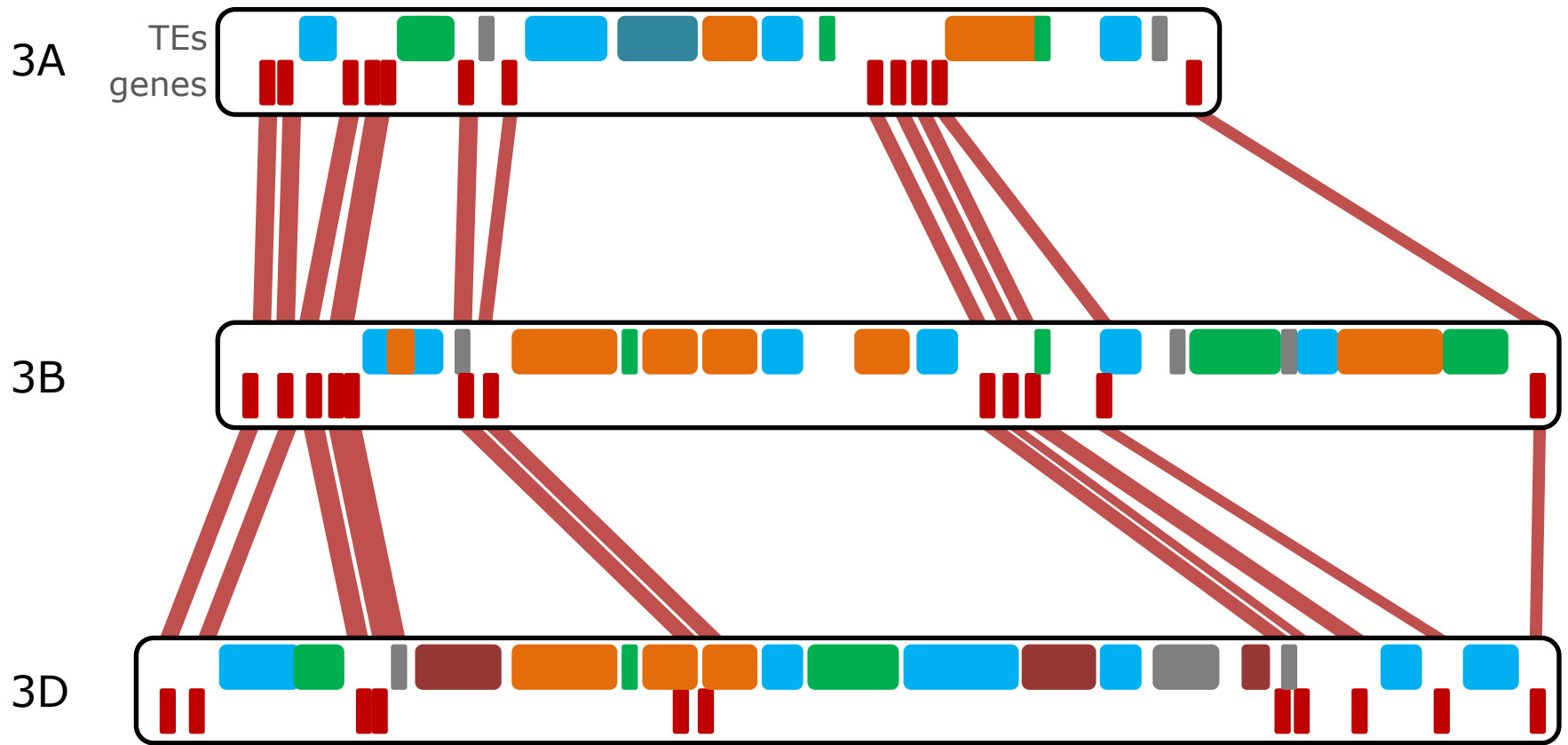


**ClariTeRep**

~500 families



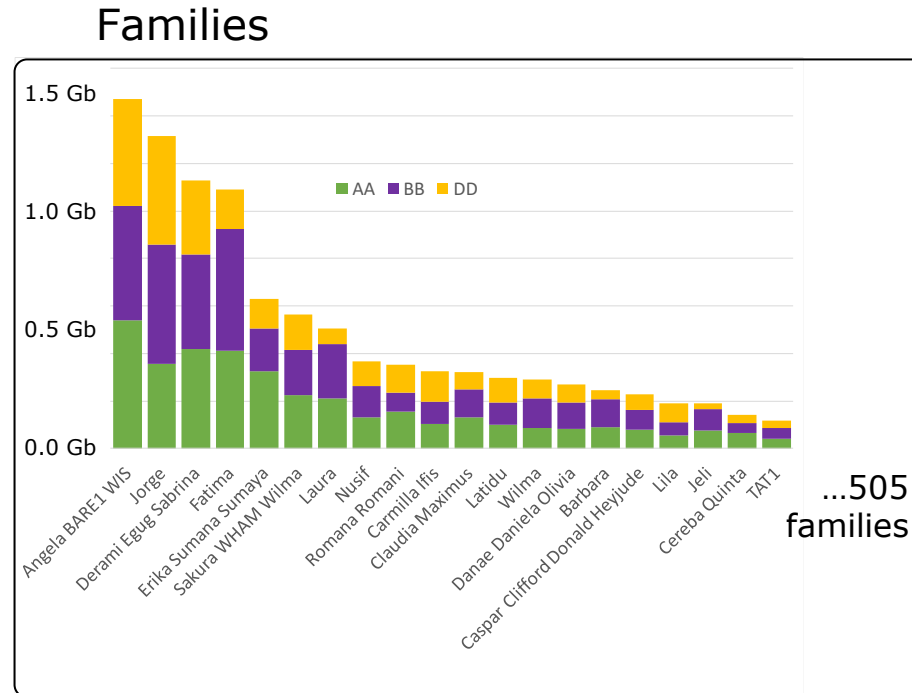




➤ Near-complete **TE turnover** since A-B-D divergence

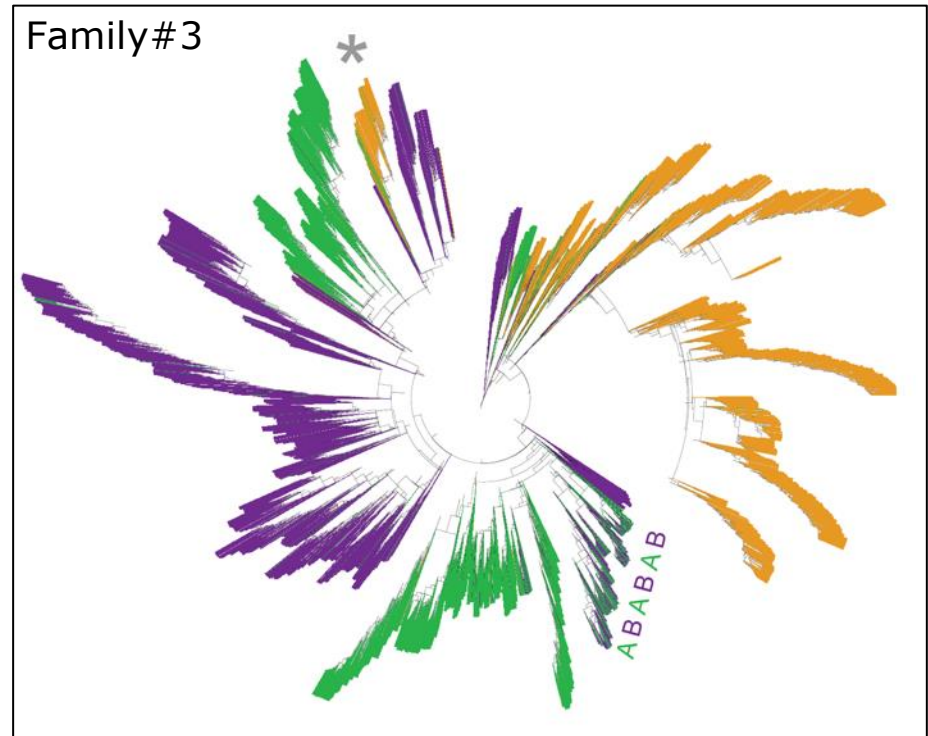
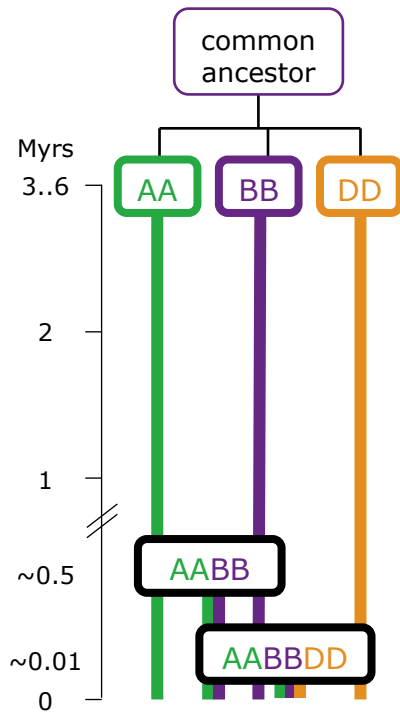


# ○ % TES



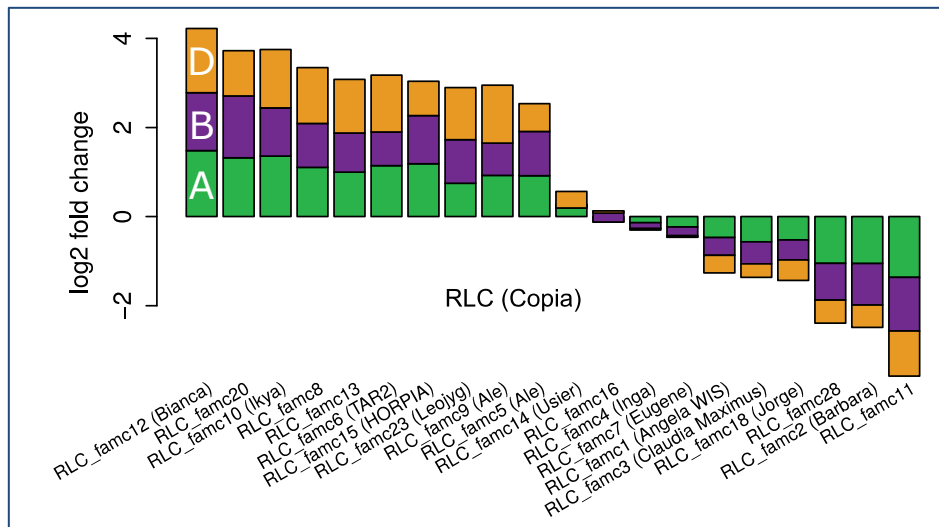
- Abundant families are the same on A-B-D
- **0** family specific to 1 subgenome
- **76%** of the fam. account for similar % on A-B-D (<2 fold-change)

# ○ TE trees

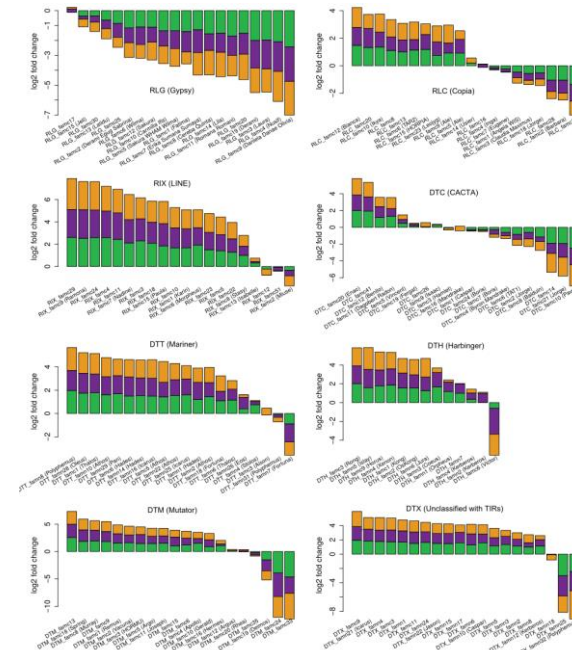


- Independent TE evolution in the diploids AA, BB, DD
- Some rare cases of TE amplif in the tetraploid AABB

# ○ TE content around genes



- Wide majority of TE families either enriched/underrepres. in gene promoters
- Enrichment/Underrepres. close to genes is conserved betw A-B-D



# ○ TE dynamics

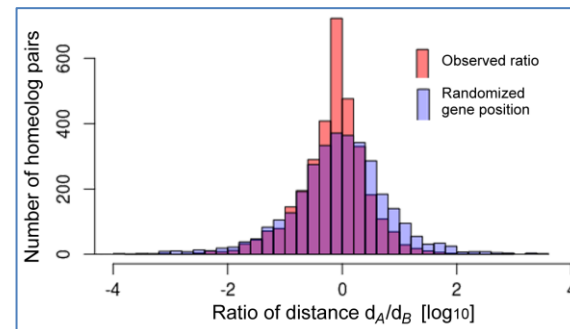
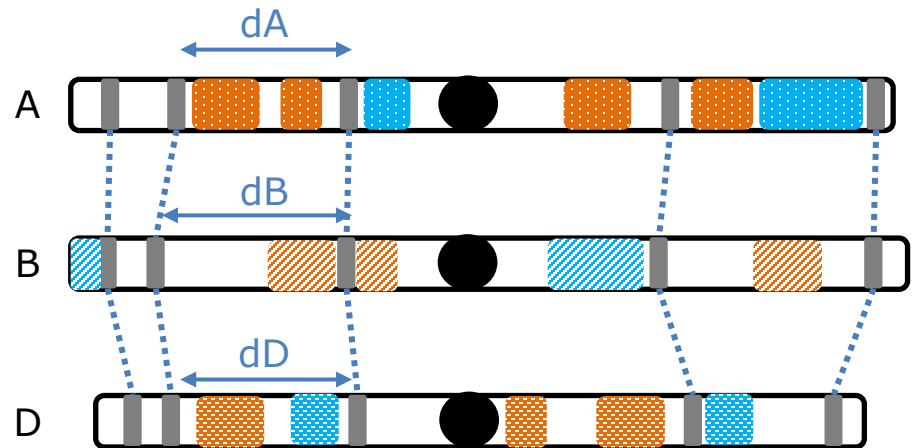
- Most families were active
- **Equilibrium**

-> Hypothesis of a structural role of TEs likely under selection pressure?

A-B-D last common ancestor

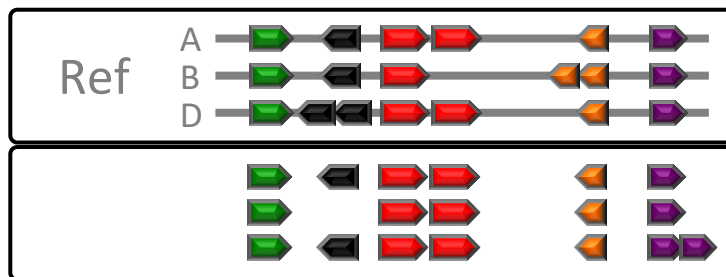


~ 3 Myrs  
TE turnover



# 4. Structural Variations in *Triticum* using resequencing data

*PhD Romain De Oliveira*



Frontiers in Genetics | www.frontiersin.org

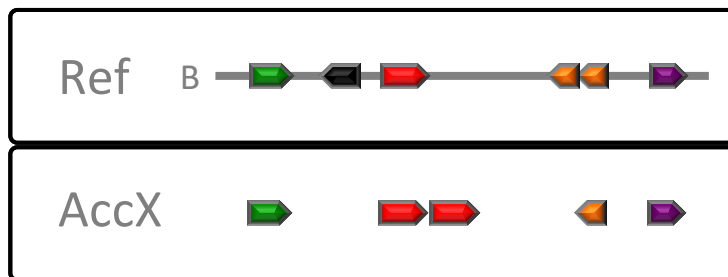
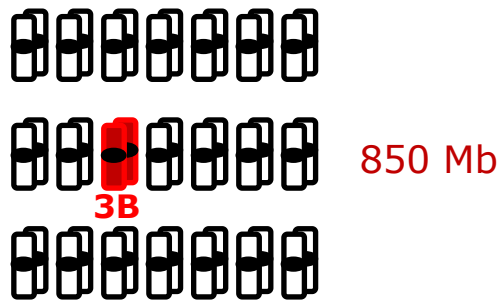
August 2020 | Volume 11 | Article 891

## Structural Variations Affecting Genes and Transposable Elements of Chromosome 3B in Wheats

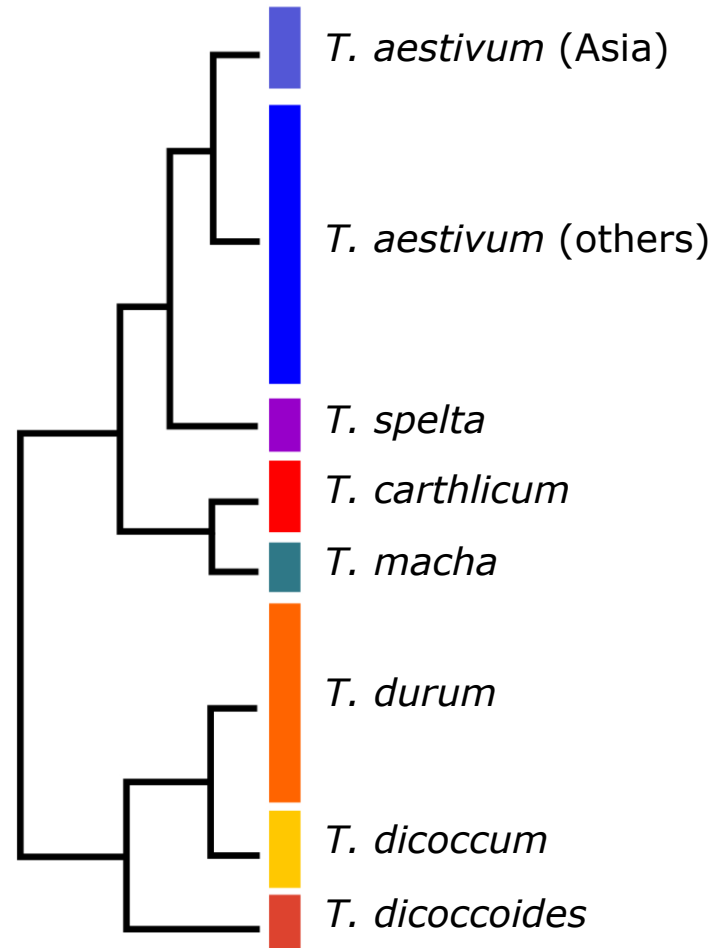
Romain De Oliveira<sup>1</sup>, Hélène Rimbert<sup>1</sup>, François Balfourier<sup>1</sup>, Jonathan Kitt<sup>1</sup>, Emeric Dynomant<sup>1</sup>, Jan Vrána<sup>2</sup>, Jaroslav Doležel<sup>2</sup>, Federica Cattonaro<sup>3</sup>, Etienne Paux<sup>1</sup> and Frédéric Choulet<sup>1\*</sup>

**45** accessions / 7 species

-> sorted 3B DNA



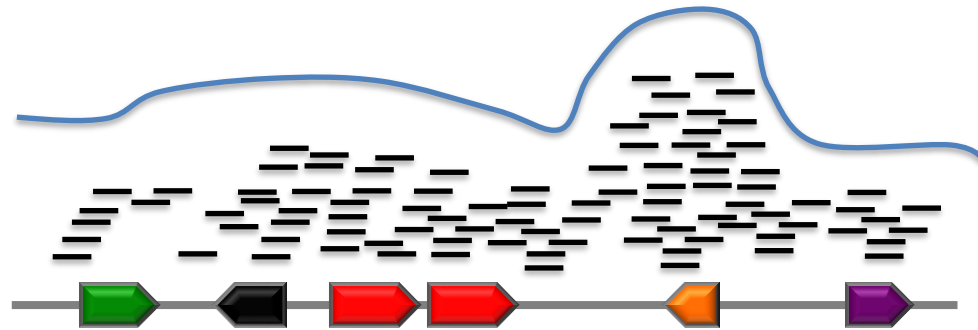
Illumina reseq ~30x



AccessionX



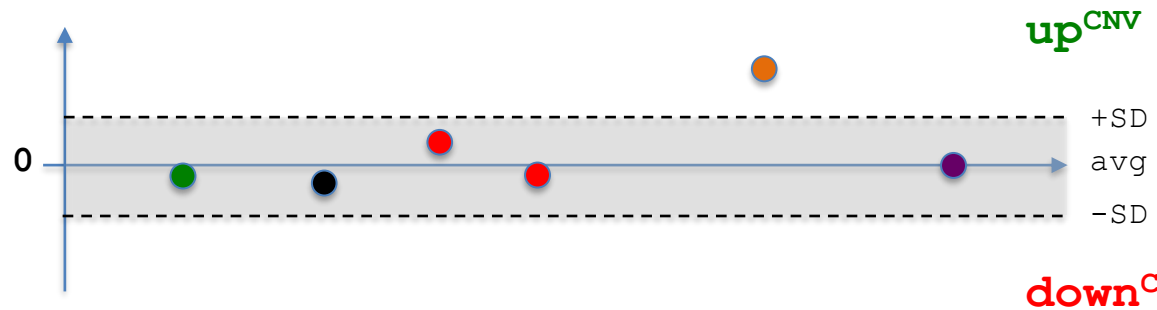
Chinese Spring

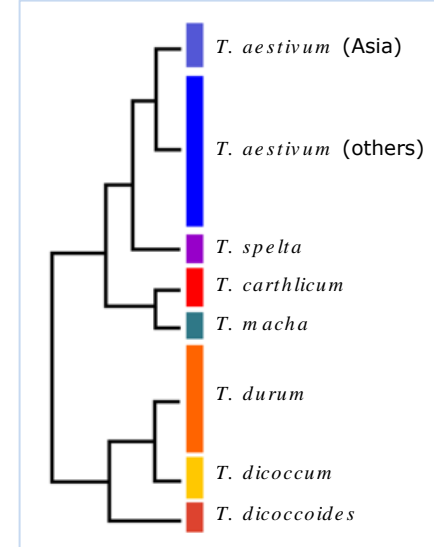
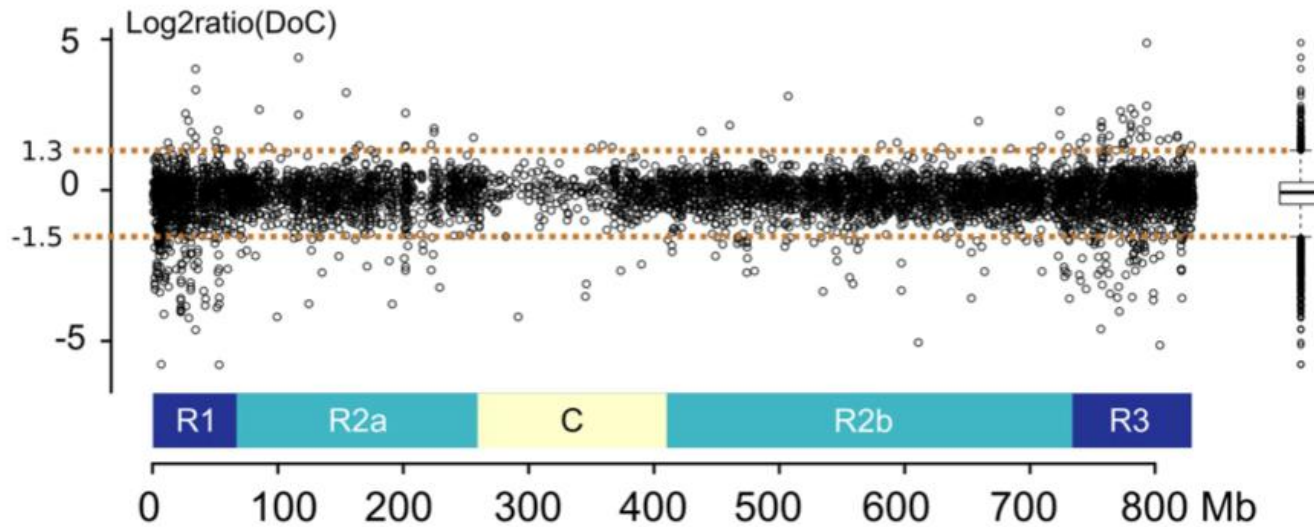


-0.1 -0.2 0.3 -0.1 1.5 0

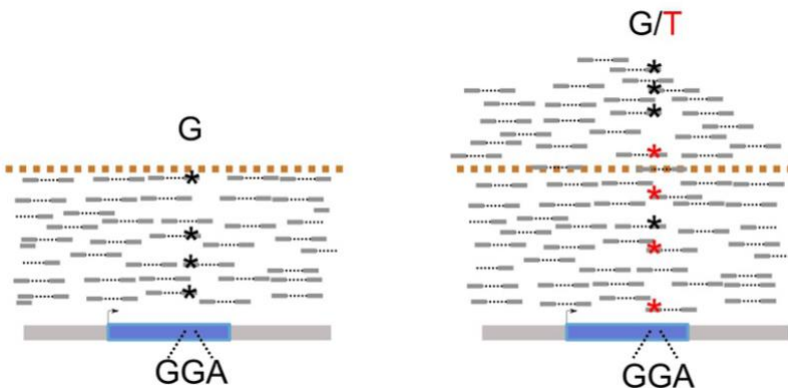
$$\log_2 \frac{\text{cov}^{\text{accX}}}{\text{cov}^{\text{ChineseSpring}}}$$

CNVs





### Chromosome 3B



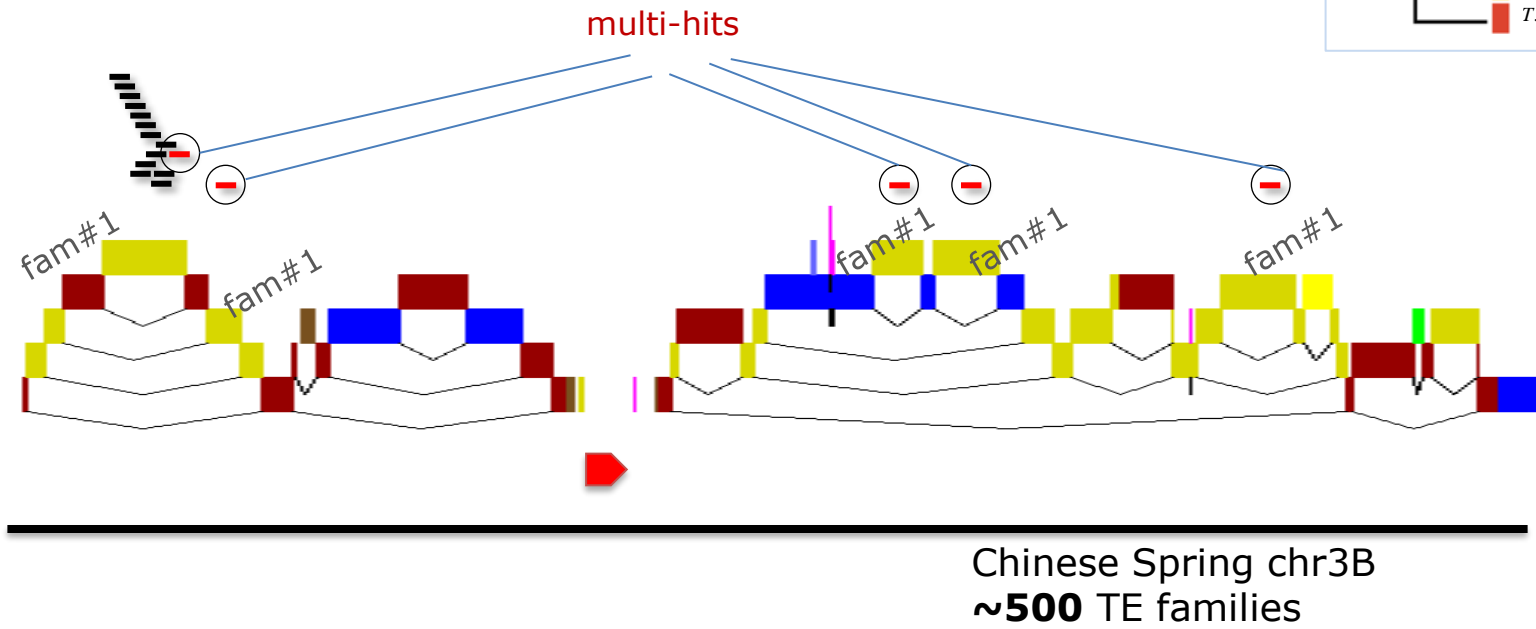
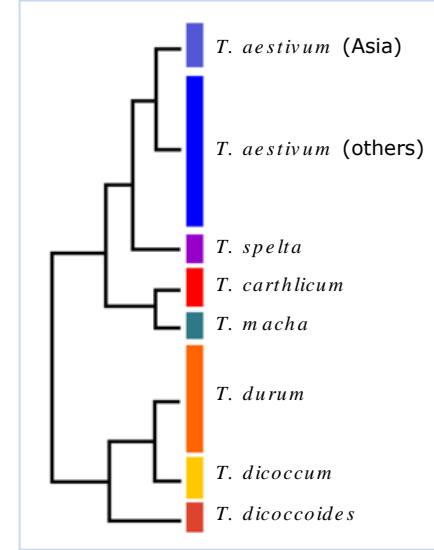
~7000 genes

PAVs/CNVs: 20% core=80%  
new genes: 330

- functions: related to stress response
- expressed in more specific conditions

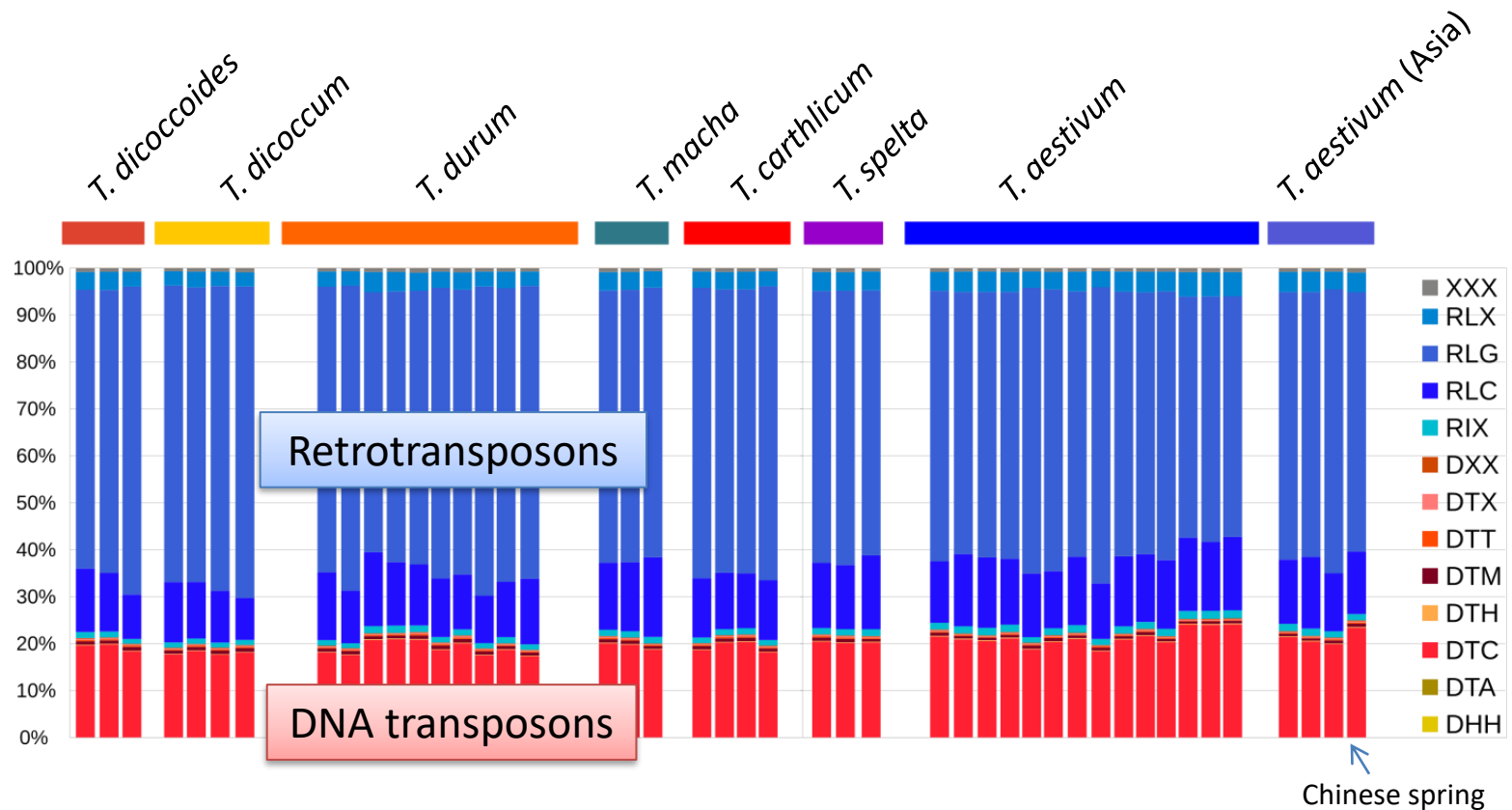


# ○ TE CNVs



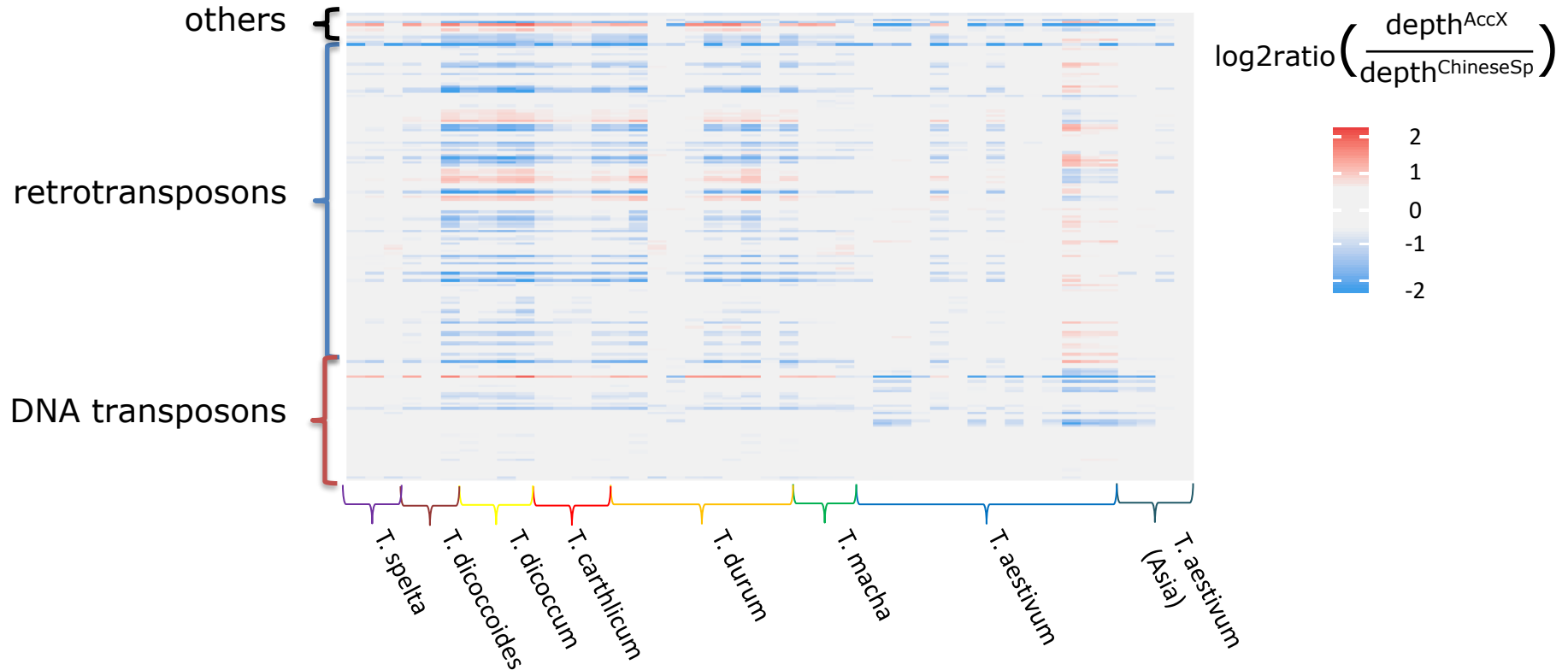
# ○ TE CNVs

TE% = **84%** in all 45 accessions



➤ No major difference of the number of TE copies for each superfamily

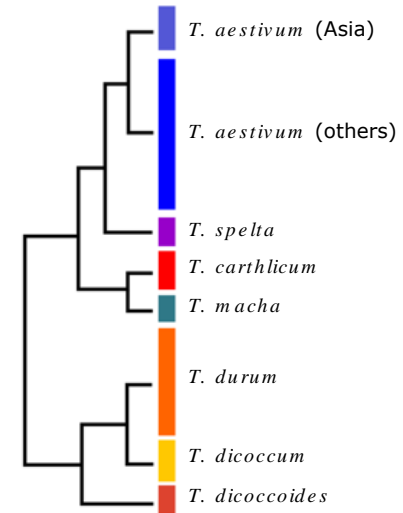
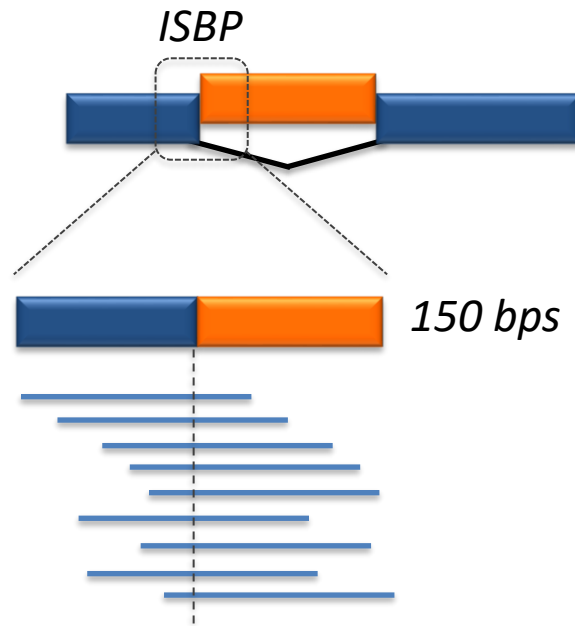
# ○ TE CNVs



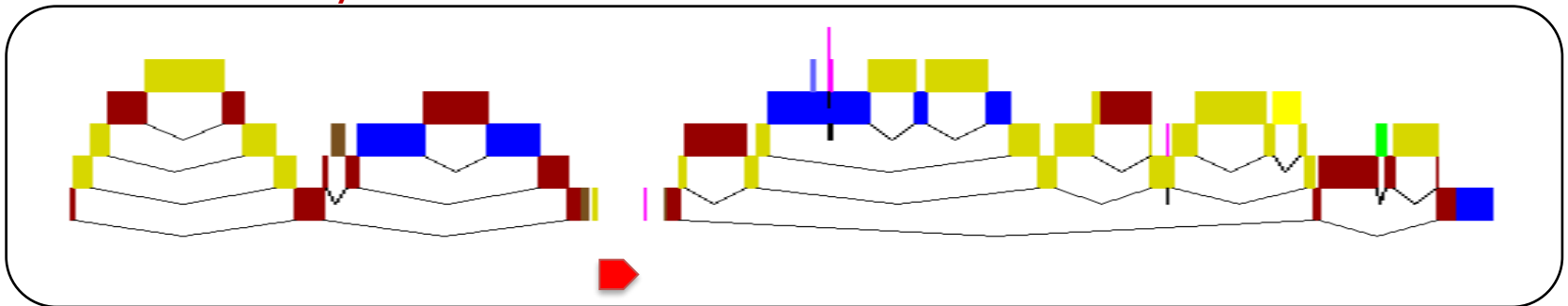
0 fam with  $\log_2\text{ratio} > 2$

➤ At the family level: globally extremely conserved proportions

# ○ TE PAVs



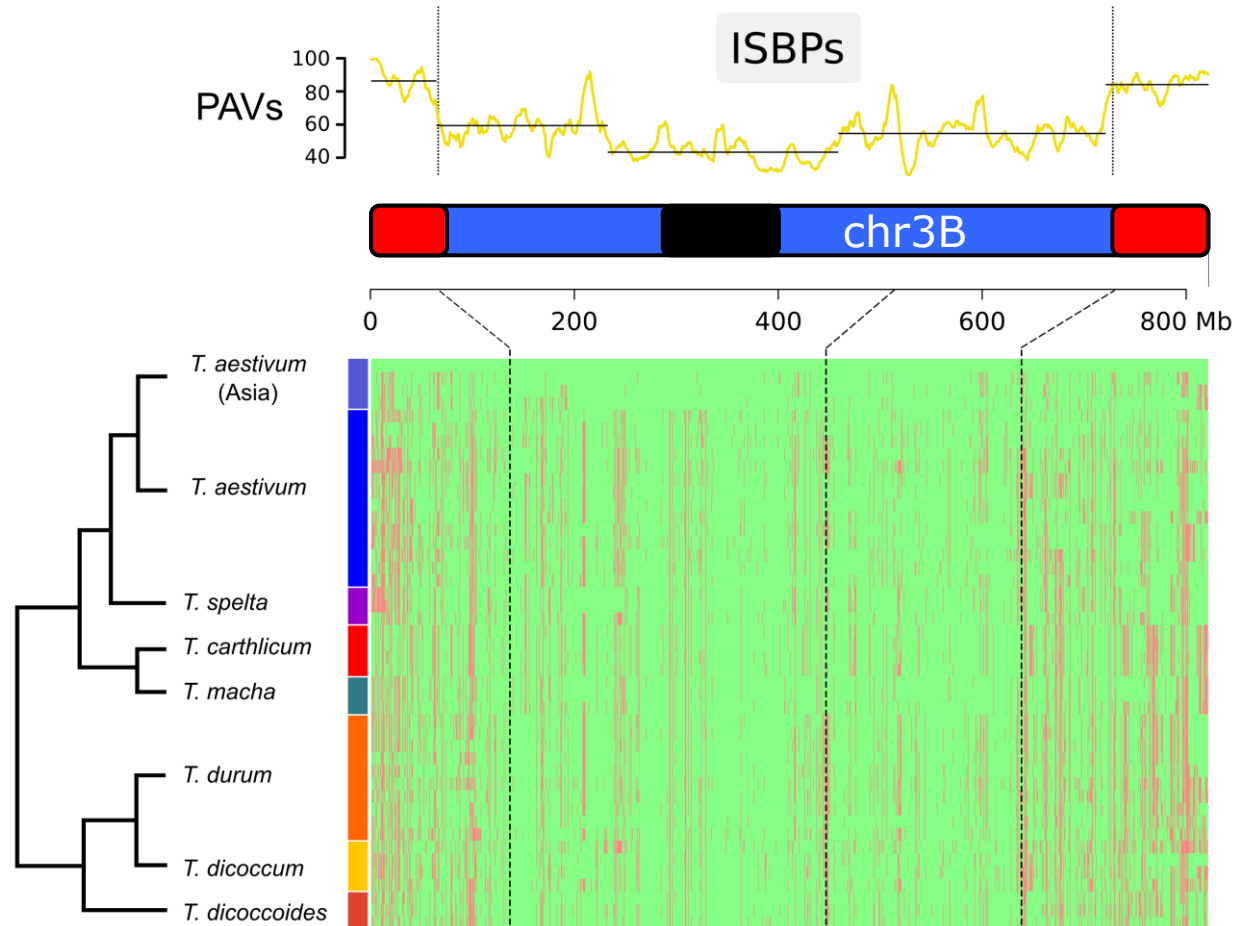
1 ISBP every 3 kb



RESULTS for 45 *Triticum* accessions:

- from **7%** to **19%** TE-PAVs
- cumulated variable: **60%** ISBPs

# ○ TE PAVs – distribution of polymorphisms



# 5. Genomic variability affecting **TEs** in wheat **using assembled genomes**

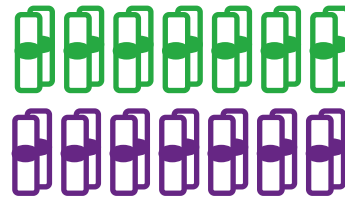
*PhD Nathan Papon (2019-2022)*

- *Methodology for comparative genomics of complex genomes with Gbps of repeats*
- *Extent of genomic variability?*
- *Active families? Amount of transposition events?*
- *Did polyploidization trigger a TE burst?*

*Triticum aestivum*



*Triticum dicoccoides*



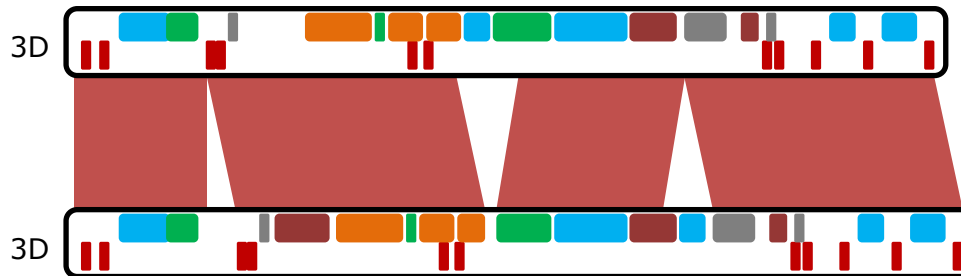
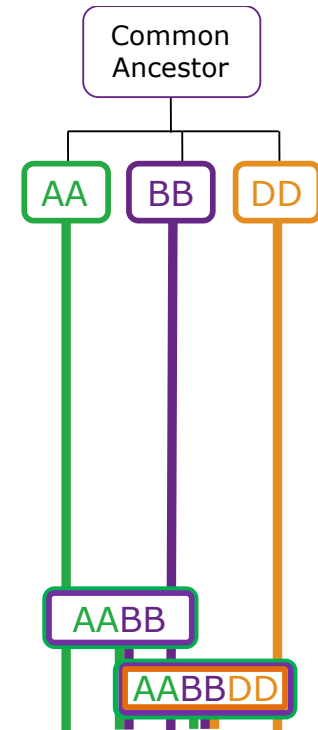
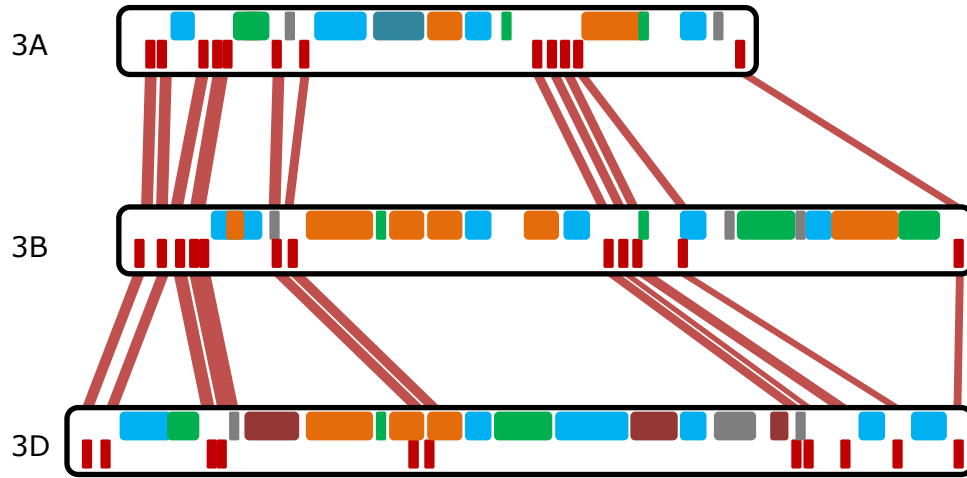
*Triticum urartu*



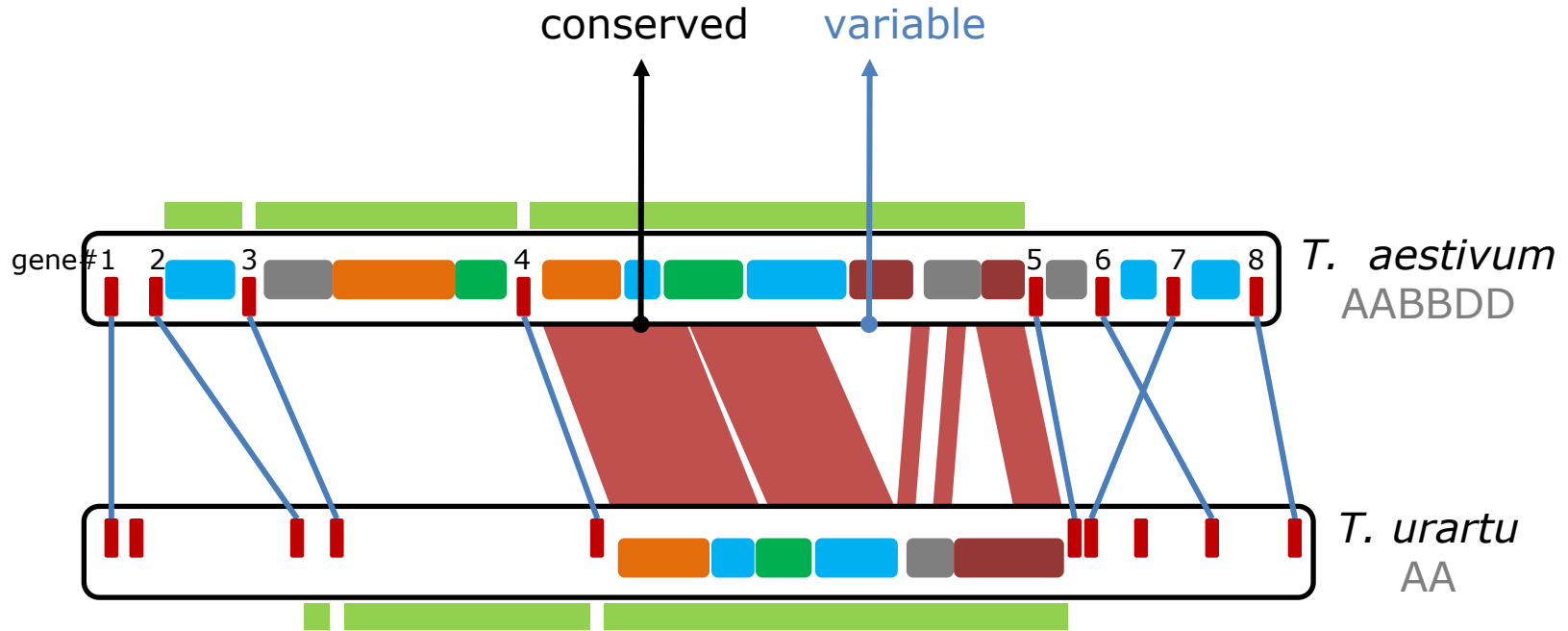
*Aegilops tauschii*



➤ Multiple RefSeq now available

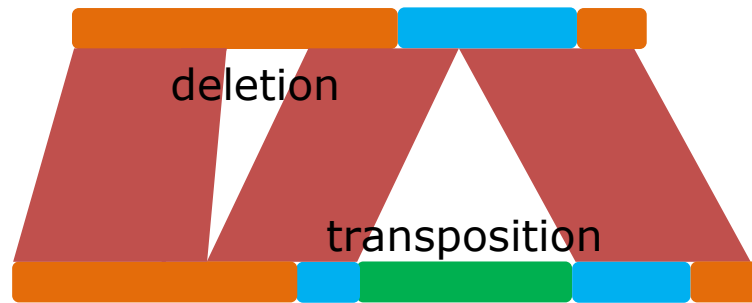






## Results:

- Variable regions represent (pairwise):
  - inter-specific: 8-34% of the sequences
  - intra-specific: 4-6% of the sequences



## Extent of variability due to recent insertions?

- ~5k–10k transposition events / subgenome (<1% of the genome)
- 238 active families

## Impact of polyploidy?

- none

Top10 families	Recent TE insertions	
	<i>T. aestivum</i> AABBDD	<i>T. durum</i> AABB
<i>RLC_famc1</i>	3032	3239
<i>DTC_famc2</i>	177	191
<i>RLG_famc2</i>	118	133
<i>RLG_famc1</i>	259	228
<i>RLG_famc7</i>	521	537
<i>RLG_famc5</i>	67	66
<i>RLG_famc3</i>	2312	2515
<i>RLG_famc4</i>	36	27
<i>RLG_famc11</i>	157	168
<i>RLG_famc10</i>	104	106

- Equilibrium

# 6. Sequencing the Renan genome



GigaScience, 2022, 11, 1–18

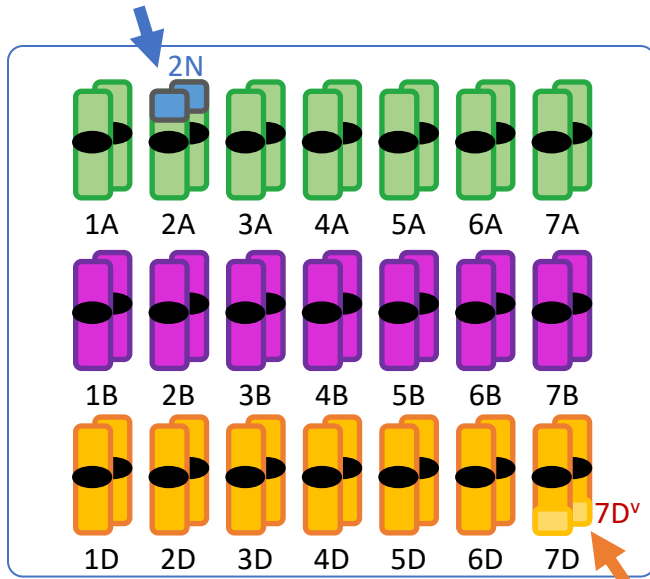
DOI: 10.1093/gigascience/giac034

RESEARCH

## Long-read and chromosome-scale assembly of the hexaploid wheat genome achieves high resolution for research and breeding

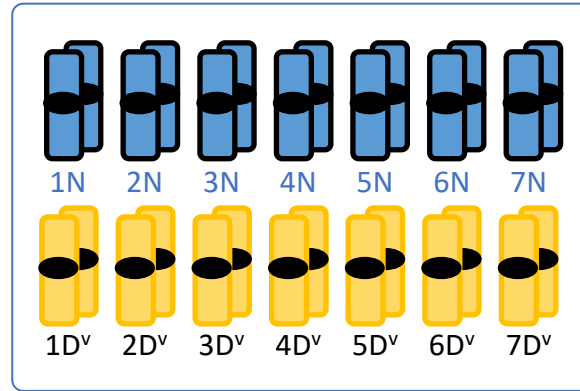
Jean-Marc Aury<sup>1,\*</sup>, Stefan Engelen<sup>1</sup>, Benjamin Istace<sup>1</sup>, Cécile Monat<sup>2</sup>, Pauline Lasserre-Zuber<sup>2</sup>, Caroline Belser<sup>1</sup>, Corinne Cruaud<sup>3</sup>, Hélène Rimbart<sup>2</sup>, Philippe Leroy<sup>2</sup>, Sandrine Arribat<sup>4</sup>, Isabelle Dufau<sup>4</sup>, Arnaud Bellec<sup>4</sup>, David Grimbichler<sup>5</sup>, Nathan Papon<sup>2</sup>, Etienne Paux<sup>2</sup>, Marion Ranoux<sup>2</sup>, Adriana Alberti<sup>1,6</sup>, Patrick Wincker<sup>1</sup> and Frédéric Choulet<sup>2,\*</sup>

Yr17 Lr37  
Sr38 Cre5

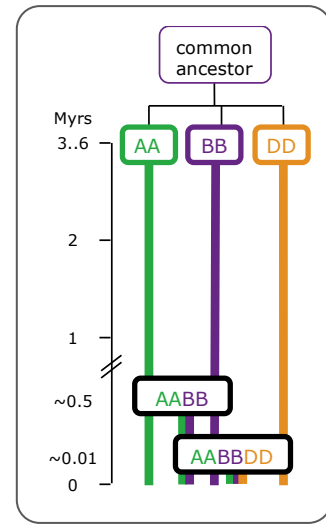


*T. aestivum*  
Renan

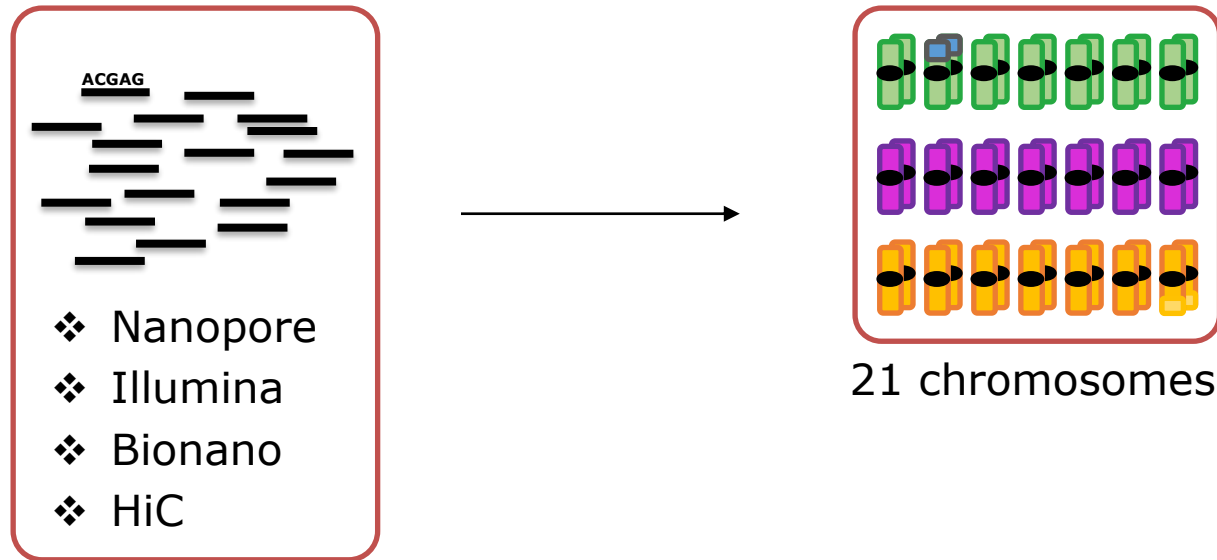
Pch1



*Ae. ventricosa*



## ○ Genome Assembly

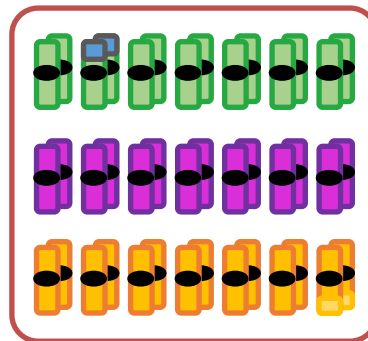


## ○ Annotation of genes

107k HC genes  
Chinese Spring



Gmap  
→

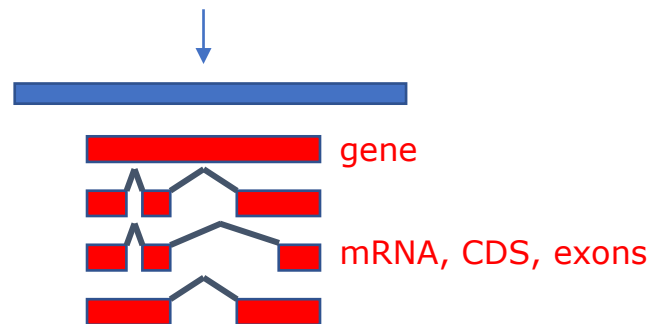
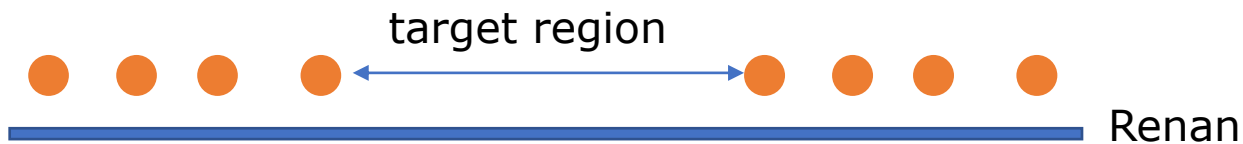
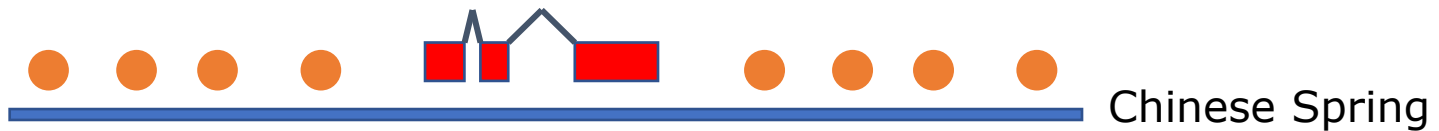
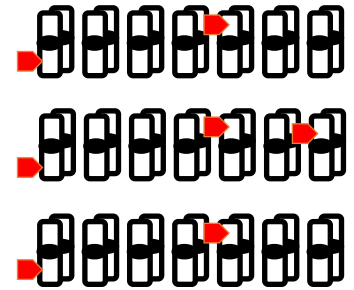
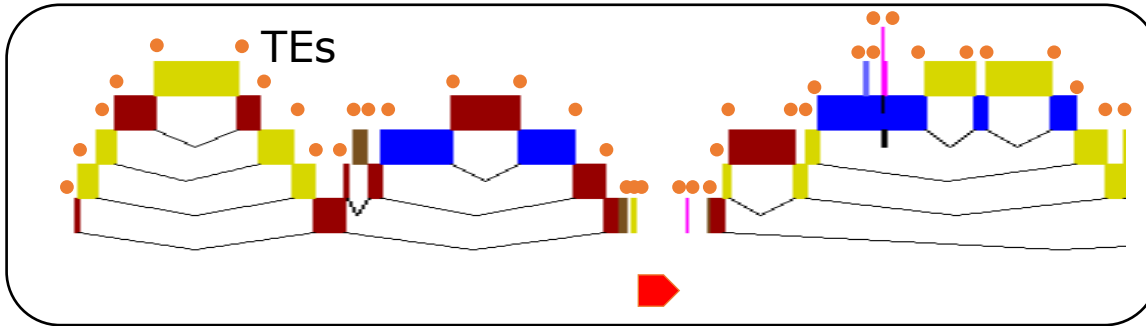


**~12% ambiguous mapping**

- *denovo* gene annotation would generate too many differences
- gene mapping from a reference is NOT satisfying
  - Develop **MAGATT**

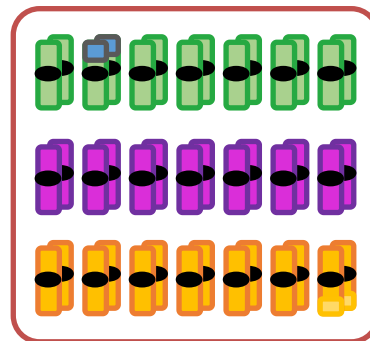
# □ **MAGATT** (**M**arker-**A**ssisted **G**ene **A**nnotation **T**ransfer for *Triticeae*)

ISBPs (uniquely mappable)



- 98% of genes mapped accurately
- + 4400 Renan specific genes

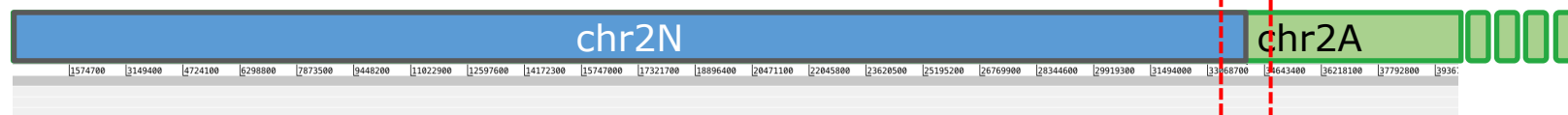
# □ *Introgessions*



telomere

Renan

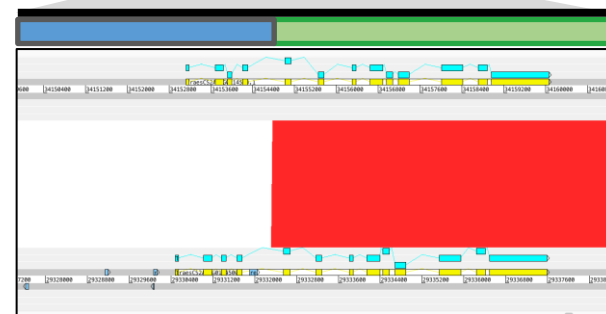
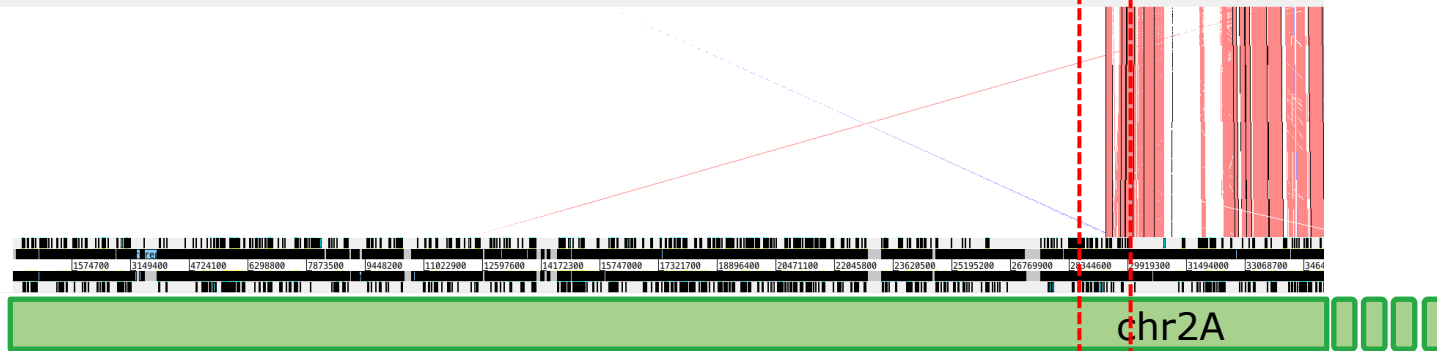
40 Mb

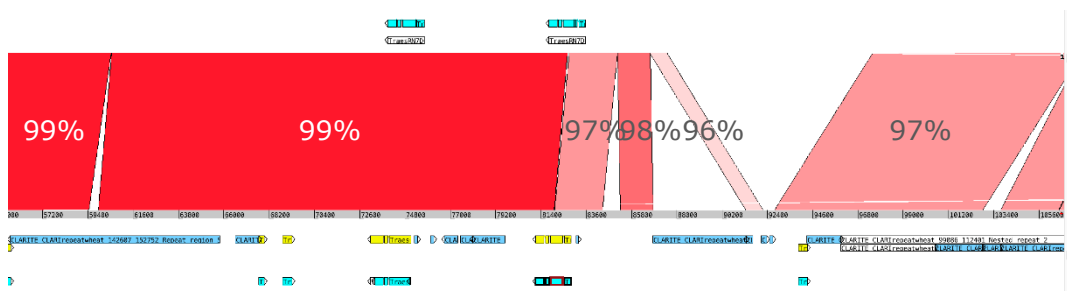
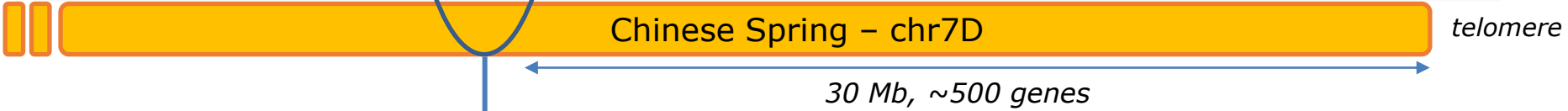
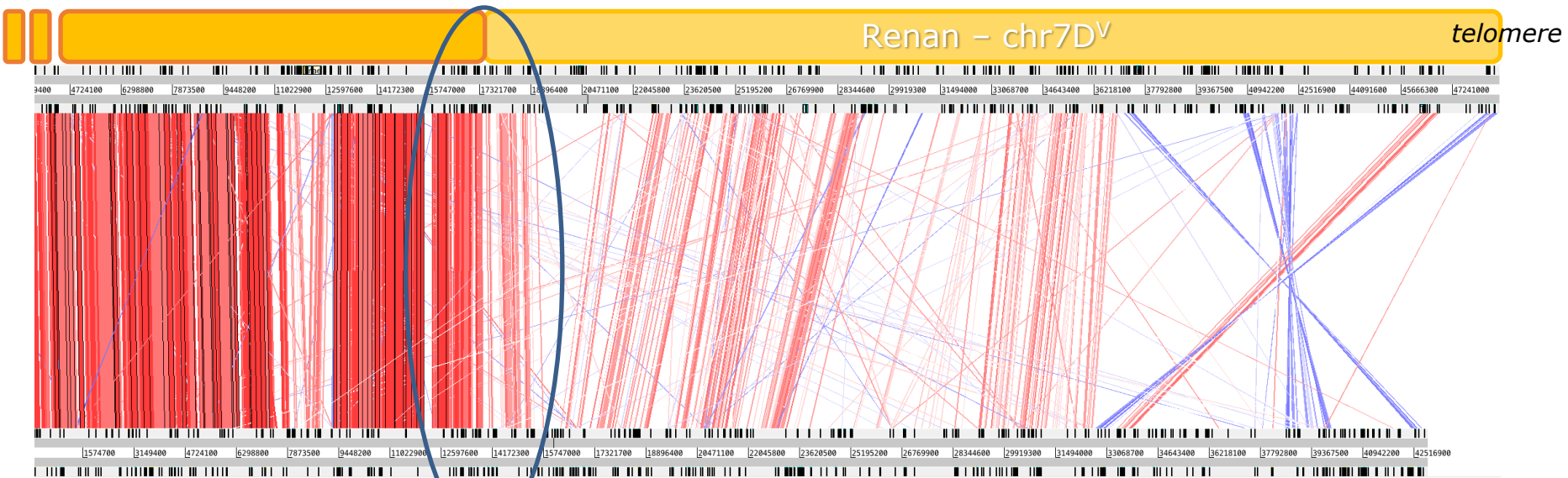


telomere

Chinese Spring

35 Mb





➤ Introgressions: source of diversity in modern breeding lines

analyses in progress...

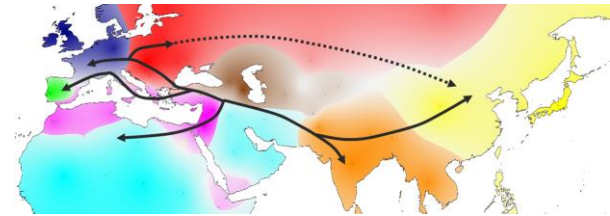
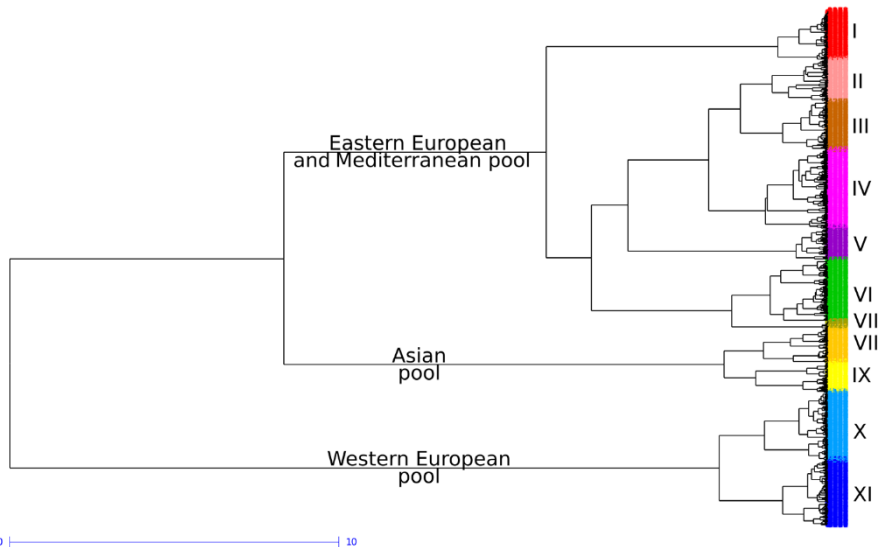


## ○ Conclusions

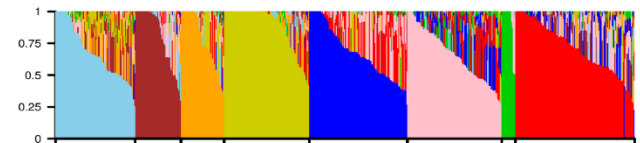
- Wheat has reached the pangenomic area
- Annotation matters

## ○ Perspectives

- Build a wheat pangenome based on 8-12 founder lines
  - IWGSC/NSF proposal under review
  - GDEC, IPS2



## STRUCTURE



# □ Acknowledgments

- *GDEC, INRAE, UCA, Clermont-Fd*

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Cécile Monat

Caroline Juery

Josquin Daron

Emeric Dynamant

Hélène Rimbart

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Jonathan Kitt

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Florence Exbrayat

François Balfourier

Etienne Paux

Pierre Sourdille

Sophie Bouchet

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Jean-Marc Aury's team (Genoscope)

Arnaud Bellec (INRAE CNRGV)

Michael Alaux (INRAE URGI)

Thomas Wicker (U Zurich)

Heidrun Gundlach (PGSB Munich)

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Federica Cattonaro (IGA, Udine)

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