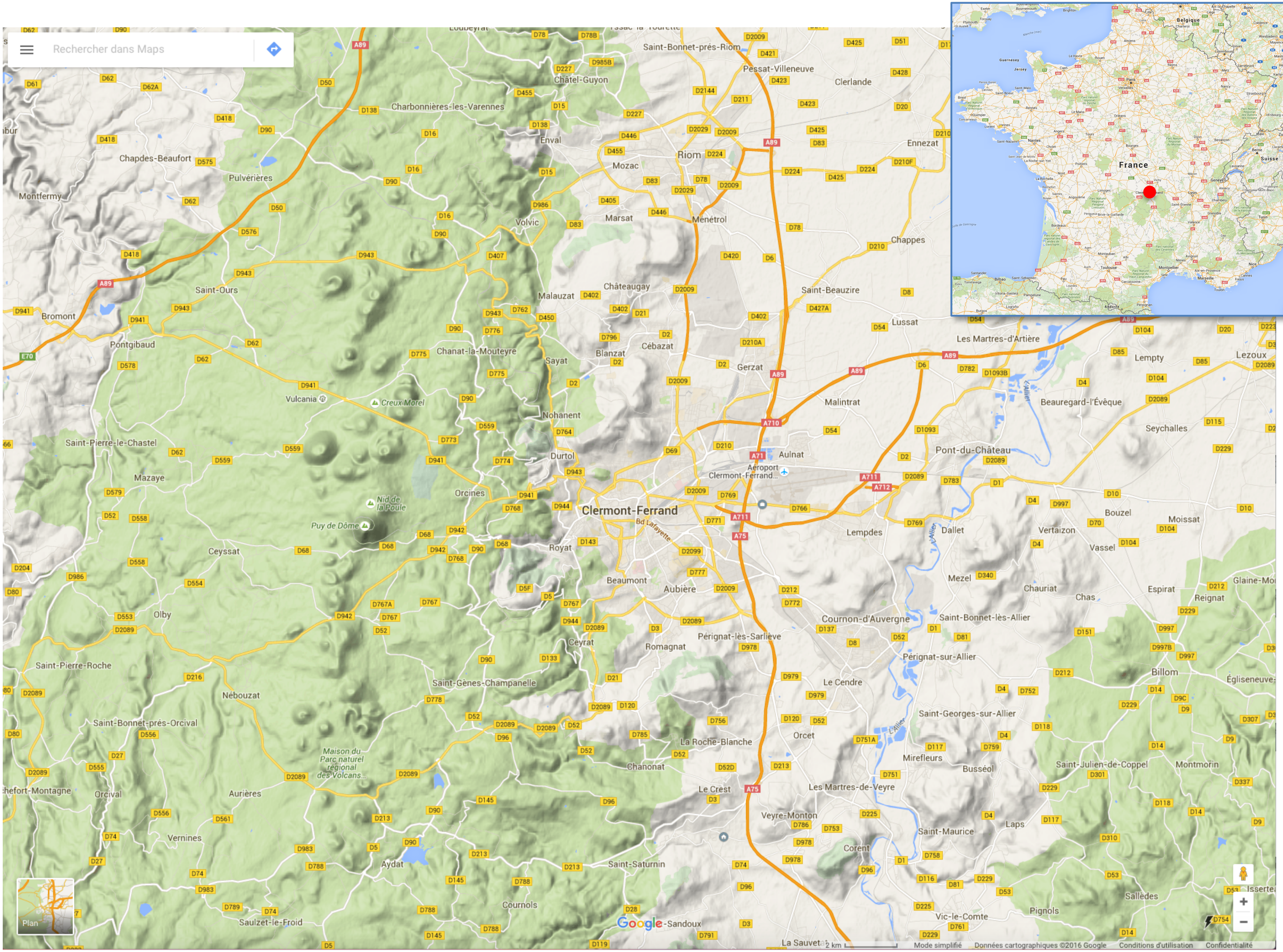
Structure and Dynamics of the Hexaploid Wheat Genome

Frédéric CHOULET

Genetics Diversity Ecophysiology of Cereals
INRA – U. Clermont-Ferrand, France



Rechercher dans Maps
Saint-Bonnet-près-Riom
Châtel-Guyon
Pessat-Villeneuve
Clerlande
Charbonnières-les-Varennes
Enval
Ennezat
Chapdes-Beaufort
Pulvérières
Riom
Mozac
Montfermy
Volvic
Marsat
Ménétrol
Chappes
Saint-Ours
Châteaugay
Malauzat
Saint-Beauzire
Bromont
Lussat
Pontgibaud
Cébazat
Blanzat
Les Martres-d'Artière
Chanat-la-Mouteyre
Sayat
Gerzat
Lempty
Lezoux
Vulcania
Creux Morel
Malintrat
Beauregard-l'Évêque
Nohanent
Seychalles
Saint-Pierre-le-Chastel
Durtol
Aulnat
Pont-du-Château
Mazaye
Aéroport Clermont-Ferrand...
Orcines
Nid de la Poule
Clermont-Ferrand
Bd Lafayette
Moissat
Bouzel
Puy de Dôme
Lempdes
Dallet
Vertaizon
Ceyssat
Royat
Vassel
Mezel
Beaumont
Aubière
Chauriat
Espirat
Glaine-Mo
Reignat
Chas
Olby
Cournon-d'Auvergne
Saint-Bonnet-lès-Allier
Ceyrat
Pérignat-lès-Sarliève
Romagnat
Pérignat-sur-Allier
Billom
Saint-Pierre-Roche
Églisneuve
Le Cendre
Saint-Genès-Champanelle
Nébouzat
Saint-Georges-sur-Allier
Saint-Bonnet-près-Orcival
Orcet
La Roche-Blanche
Maison du Parc naturel régional des Volcans...
Mirefleurs
Saint-Julien-de-Coppel
Chanonat
Busséol
Montmorin
Rochefort-Montagne
Orcival
Aurières
Le Crest
Les Martres-de-Veyre
Veyre-Monton
Laps
Vernines
Saint-Maurice
Corent
Aydat
Saint-Saturnin
Isserte
Salledes
Cournols
Pignols
Vic-le-Comte
Saulzet-le-Froid
Sandoux
La Sauvetat
Plan
Google
2 km
Mode simplifié
Données cartographiques ©2016 Google
Conditions d'utilisation
Confidentialité
France
Belgique
Suisse

GDEC
Genetics Diversity Ecophysiology of Cereals

- Structure, evolution of wheat genome
- Recombination
- Paleogenomics
- Grain composition
- Response to abiotic stress
- Resistance to pathogens
- Diversity, selection

Objectives

❑ Resources

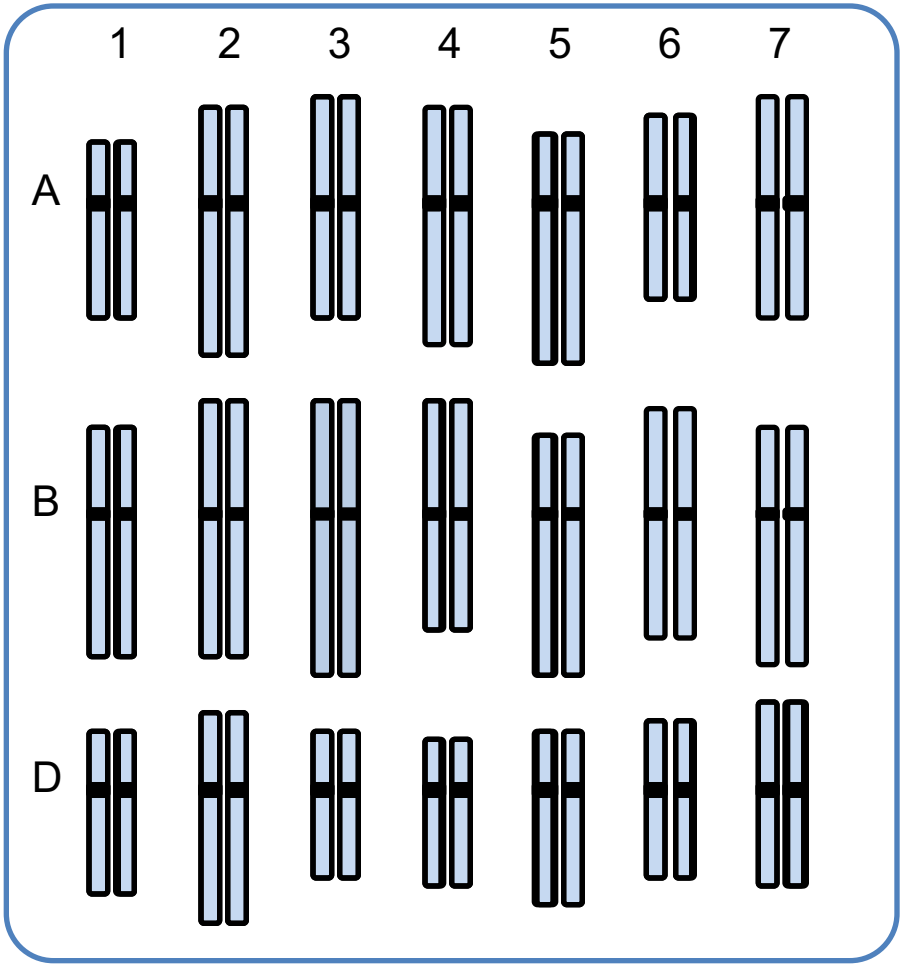
- Sequencing the wheat genome
- Markers
- Bioinformatics

❑ Research

- Structure / Expression / Evolution
  - TEs
  - Gene space
  - Duplications
  - Structural variations
  - Epigenome

❑ Why wheat?

- Important crop
- Complex genome



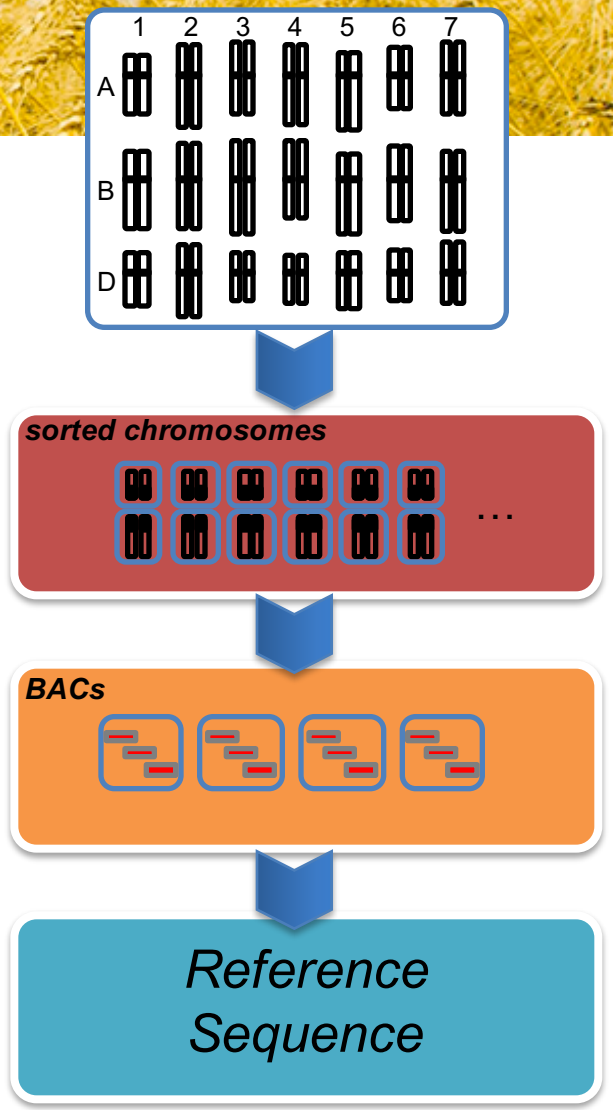
17 Gb

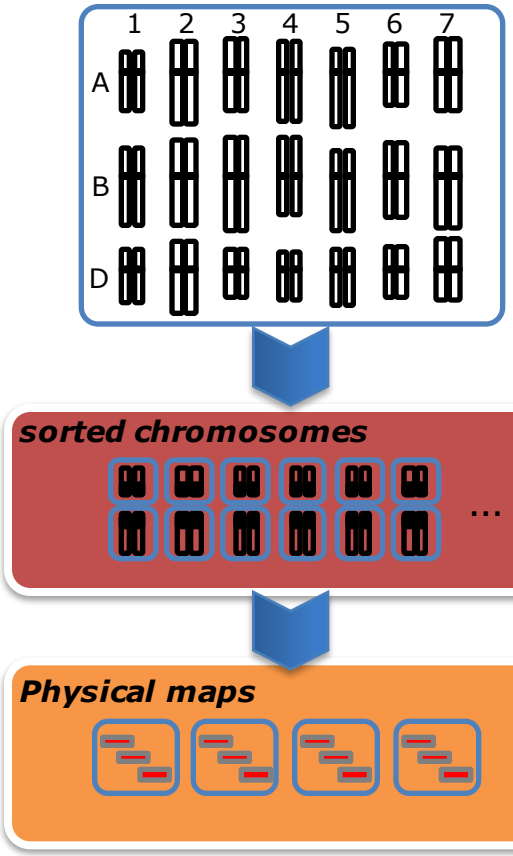
AABBDD

85% TEs

Generating a high quality genome sequence of bread wheat

- Launched in 2005
- Goal
  - ➢ *Produce a high quality ref seq of the bread wheat genome*
- Strategy
  - ➢ *Reduce the complexity*





WGS

- *Brenchley et al. Nature 2012*
- *Ling et al. Nature 2013*
- *Jia et al. Nature 2013*
- *Chapman et al. Genom Biol 2015*

Chr. Survey Seq (=CSS)

- *IWGSC Science 2014*

MTPseq

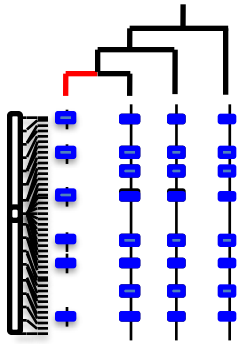
3B

- *Choulet et al. Science 2014*
- *Daron et al. Genom Biol 2015*
- *Pingault et al. Genom Biol 2015*
- *Glover et al. Genome Biol 2015*

❑ Chr Survey Seq (2014)

❑ Resources

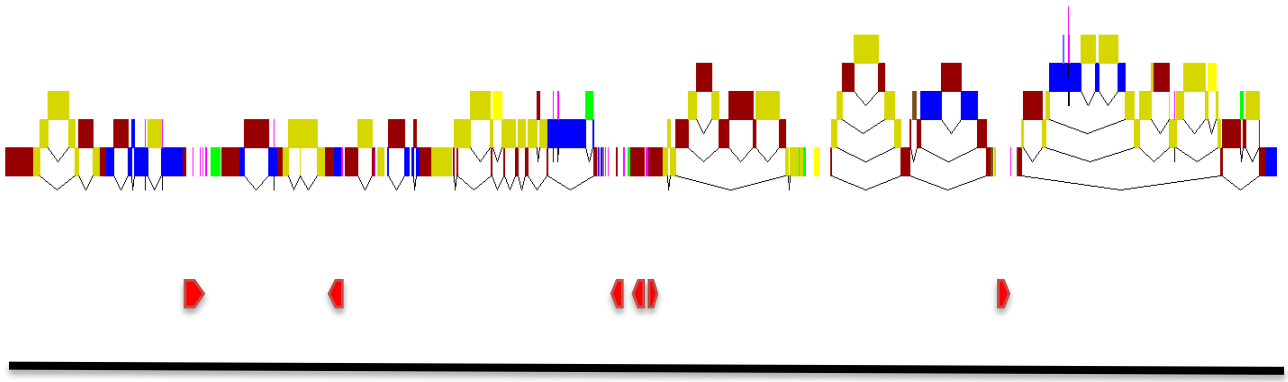
- 1 draft seq / chromosome arm
- **10** Gb - **10 M** contigs (N50: 2.4 kb)
- **99,000** genes
- ~60% genes "zipped"



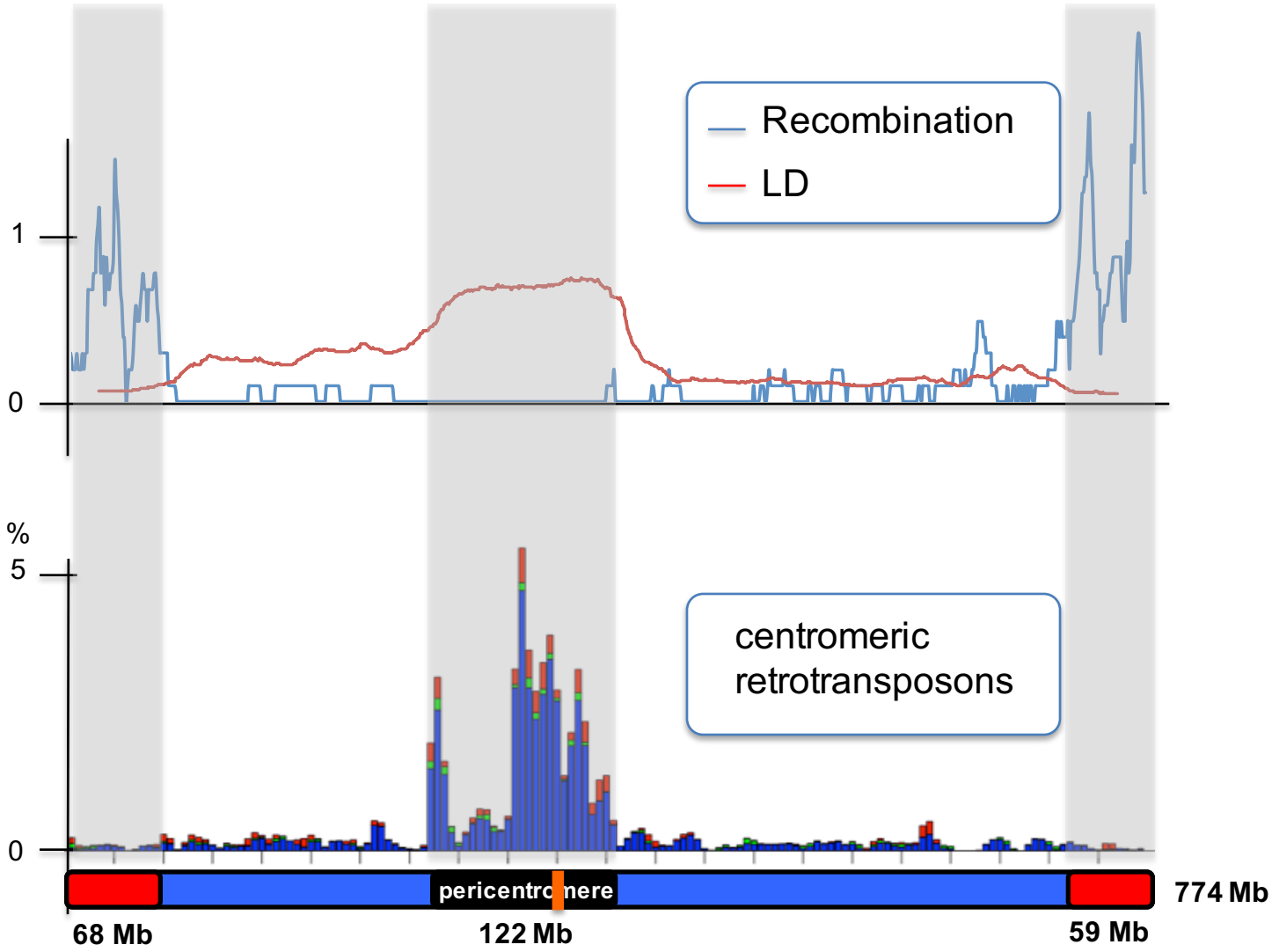
❑ Main Results

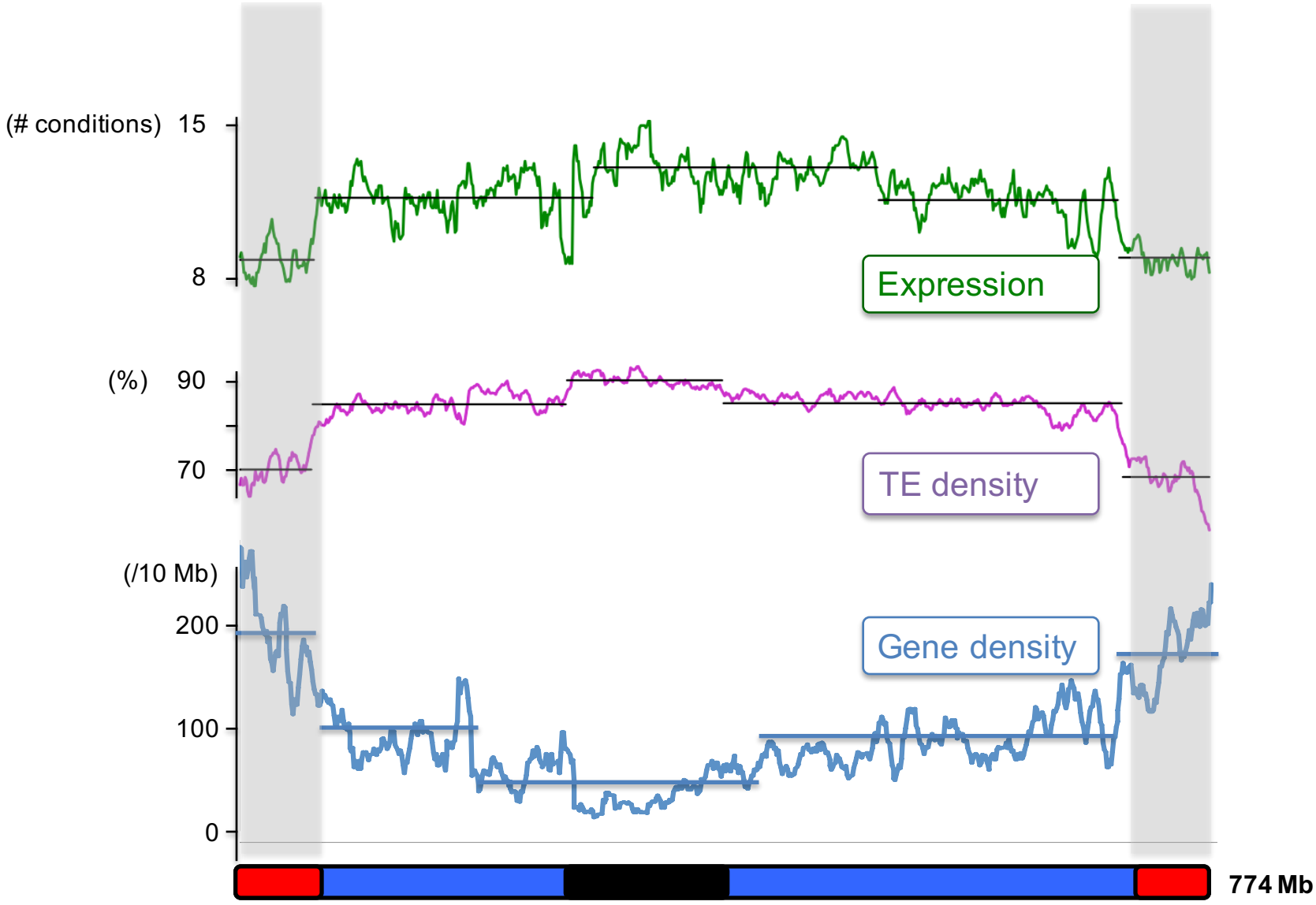
- Gene loss--
- SSD++
- Dominance--

❑ 3B pseudomolecule (3BSEQ project)

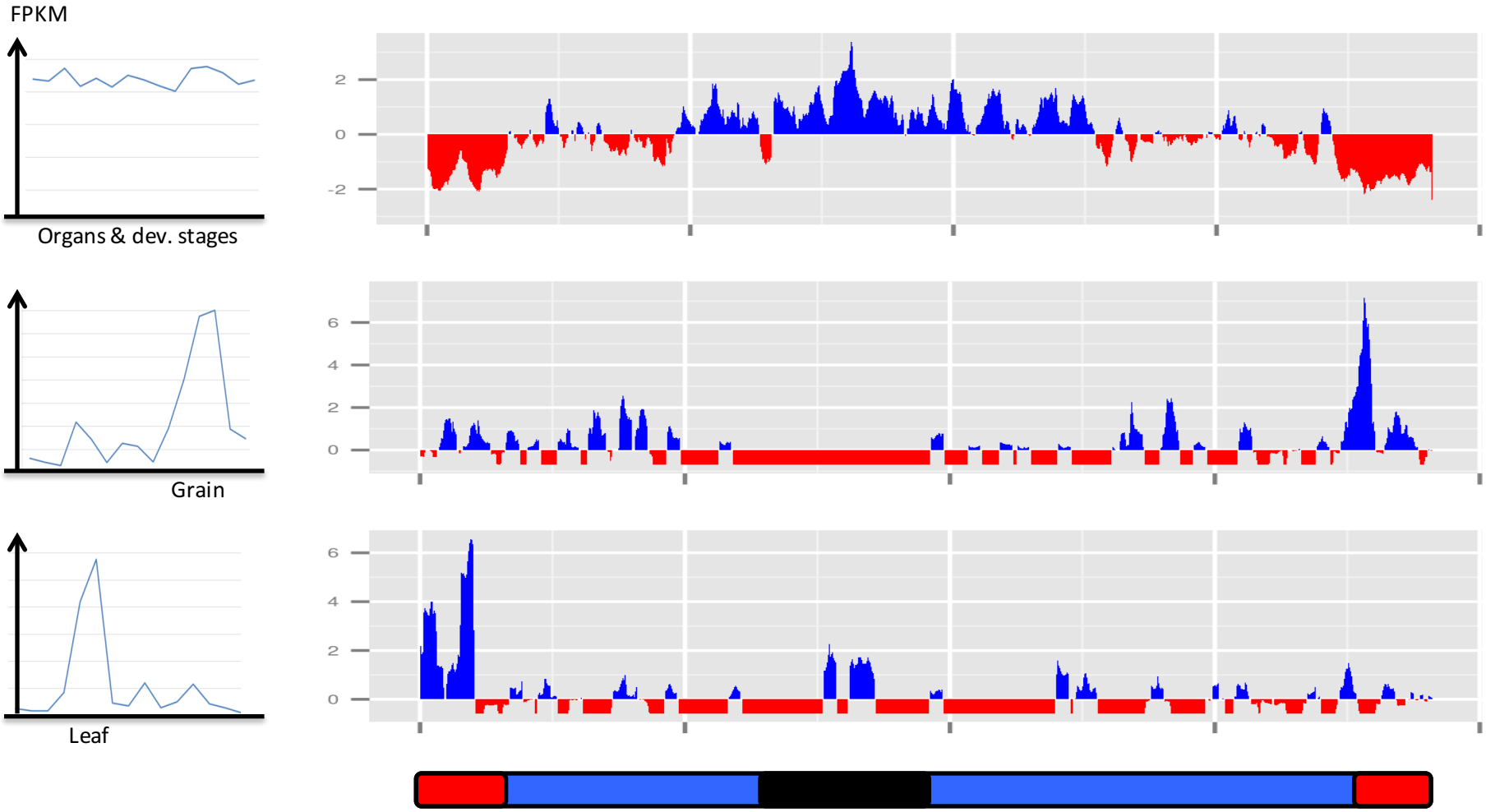


- protein coding genes 7,264
  (pseudogenes: 27%)
- transposable elements 252,879 (86%)


Recombination
LD
1
0
%
5
0
centromeric
retrotransposons
pericentromere
774 Mb
68 Mb
122 Mb
59 Mb

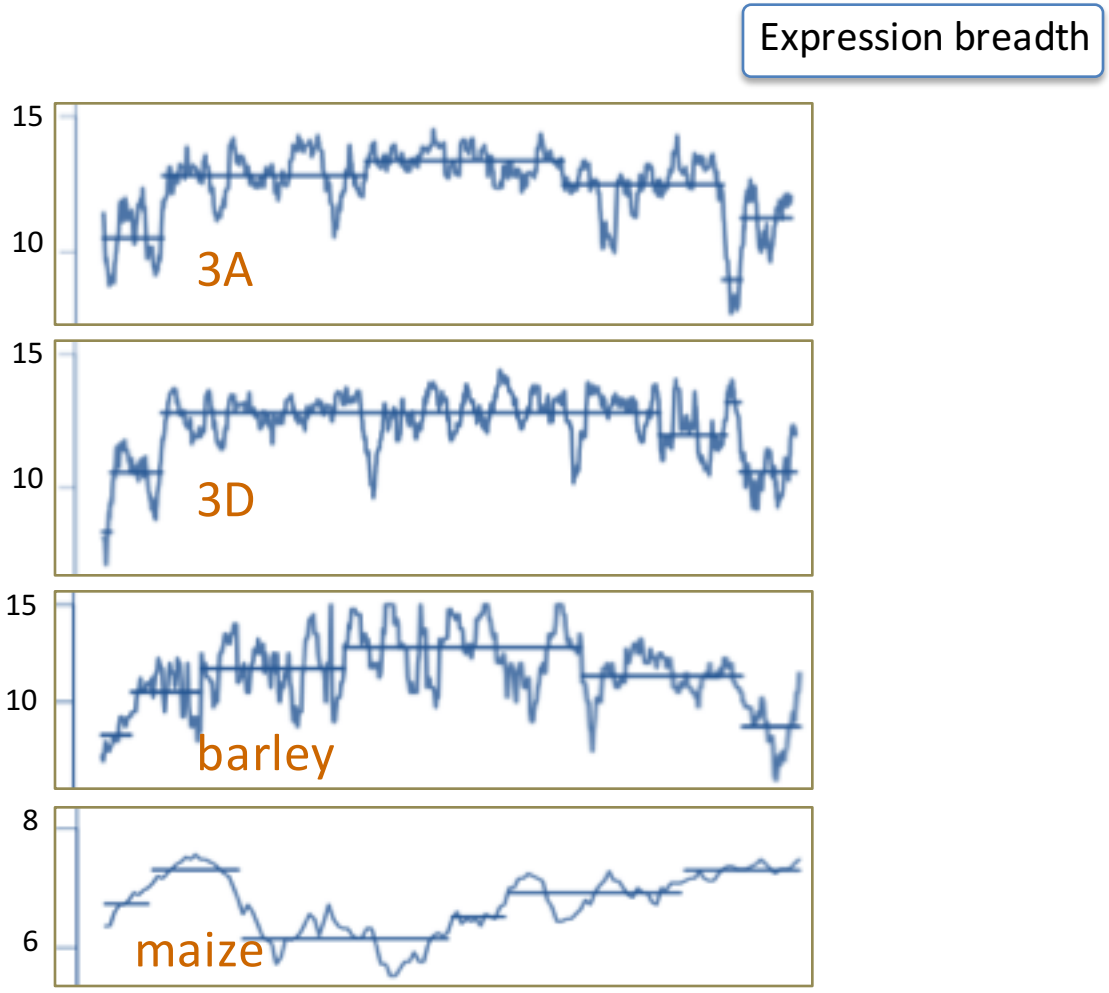


Choulet et al. Science 2014

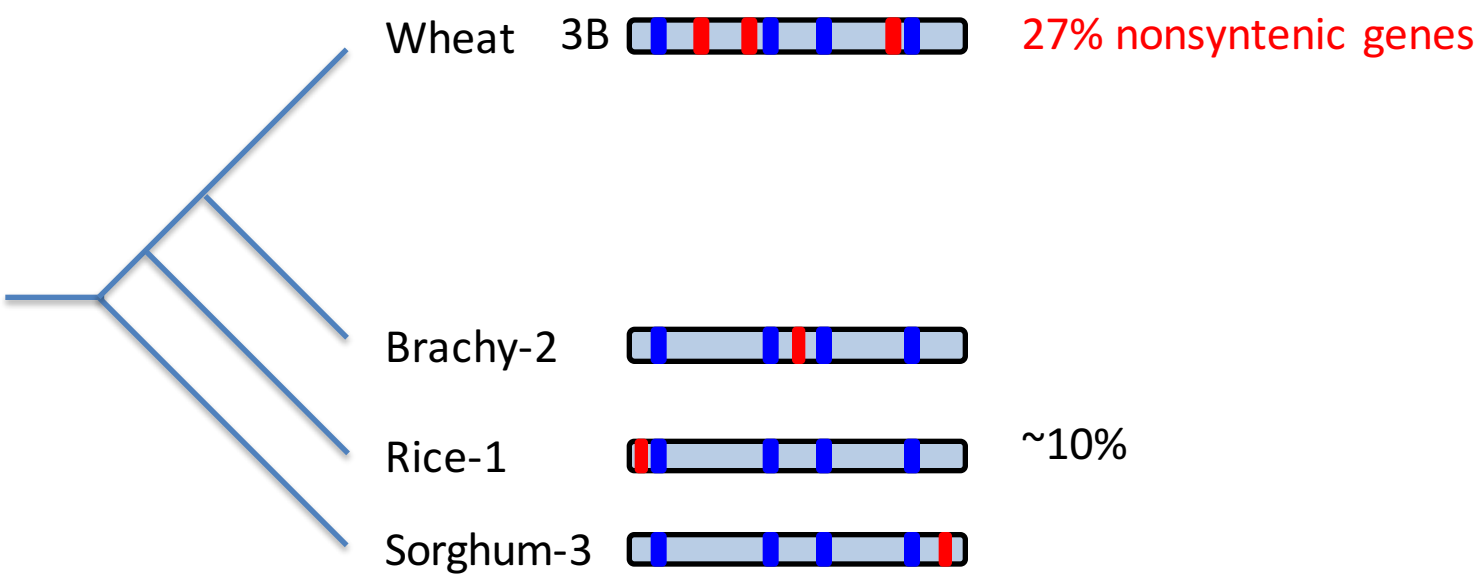


Pingault et al. Genom Biol 2015

❑ Chromosome partitioning: 3B-specific??



❑ Accelerated evolution in the *Triticeae*

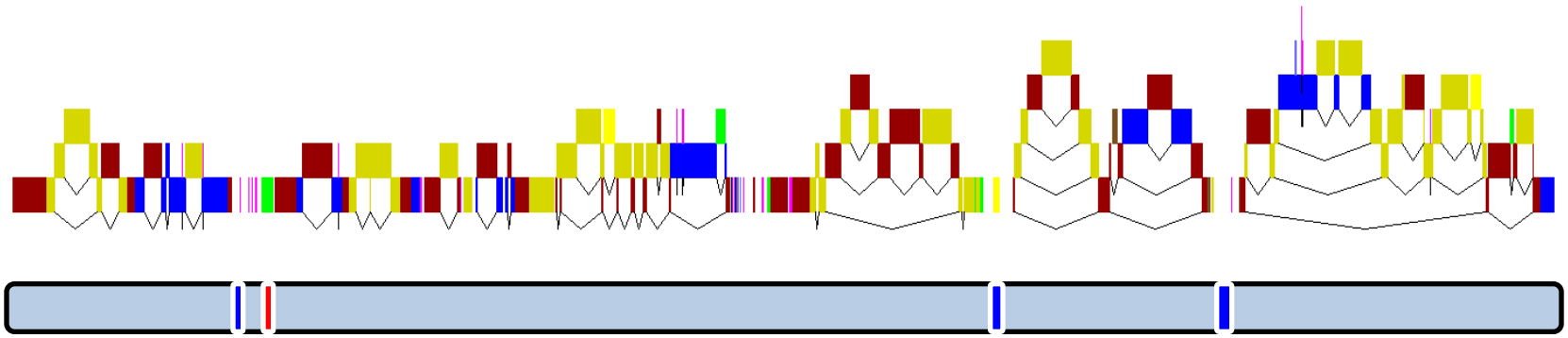


- inter-chromosomal gene duplications
- intra-chromosomal -

- More duplicated genes in the chr. extremties
- Enriched in adaptation functions

Glover et al. Genom Biol 2015

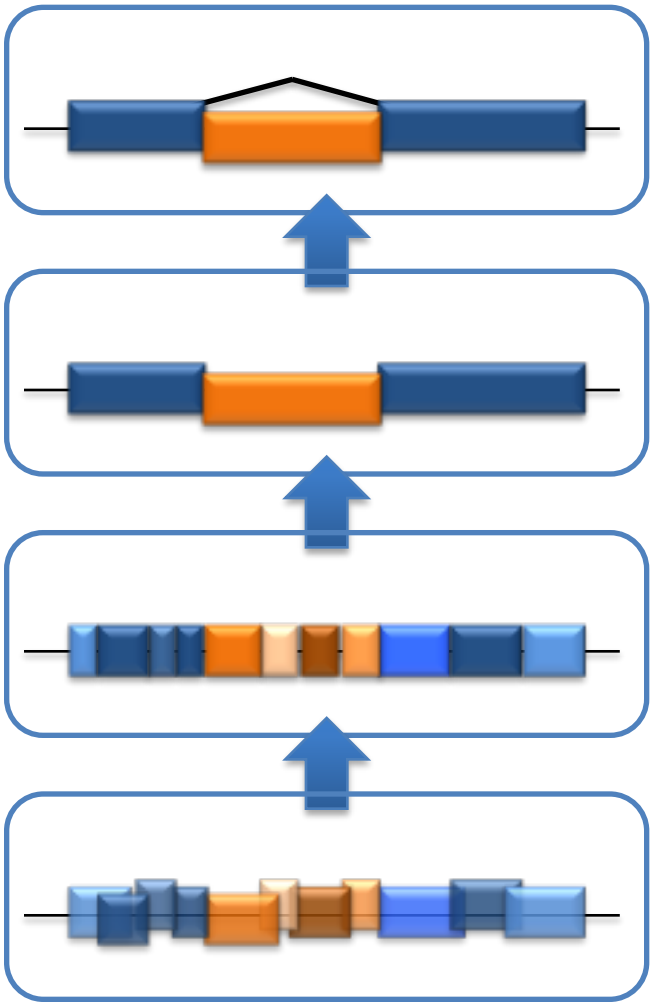
❑ TEs



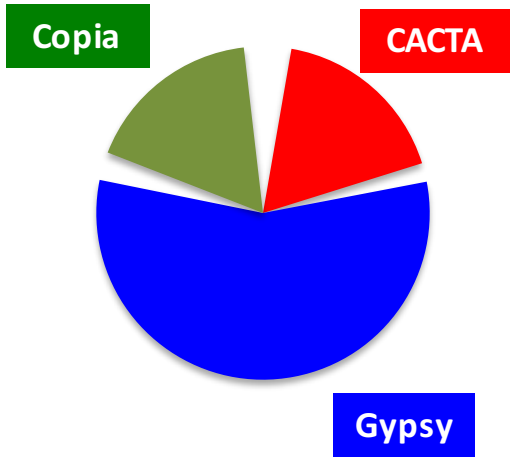
- Annotation challenge
- Impact on genome biology

Daron et al. Genom Biol 2015

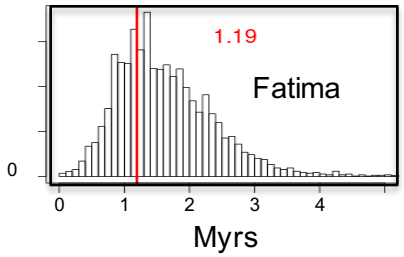
❑ New tools: **CLARITE** and **ClariTeRep**

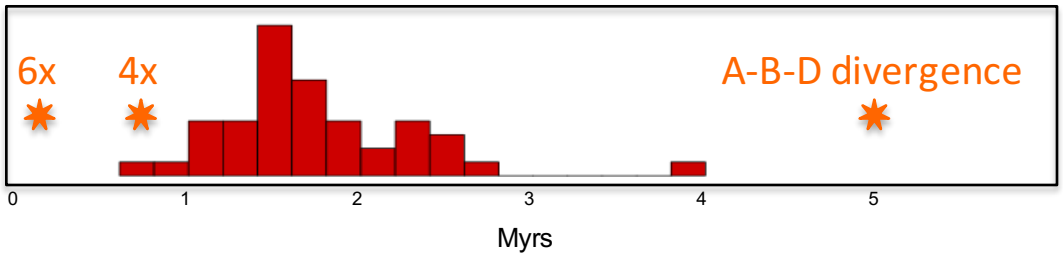


3B → ***252 000*** TEs

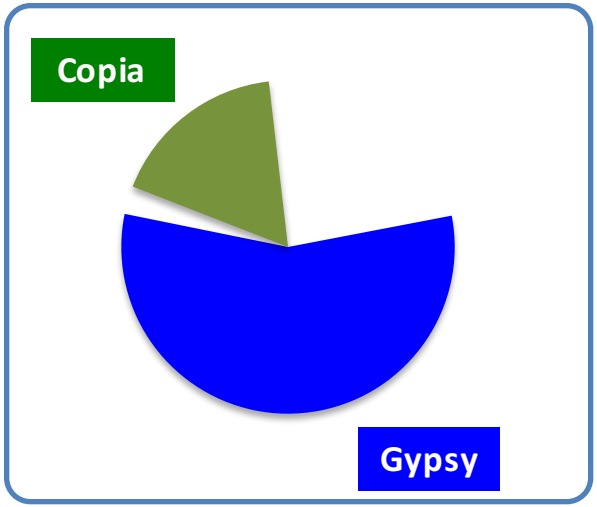


❑ Insertion dynamics

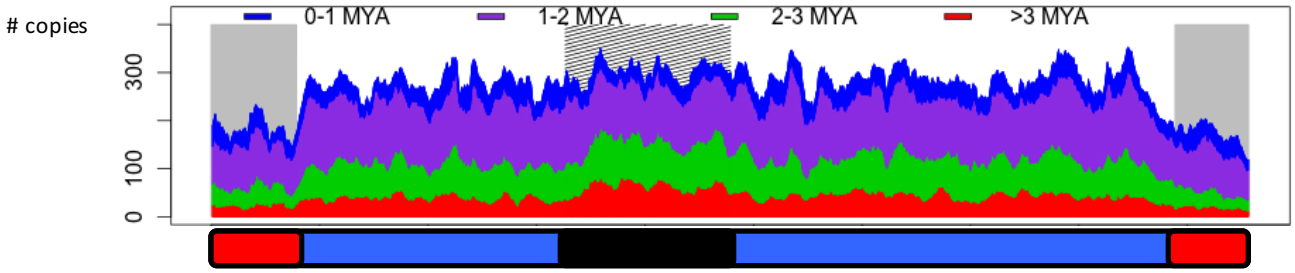


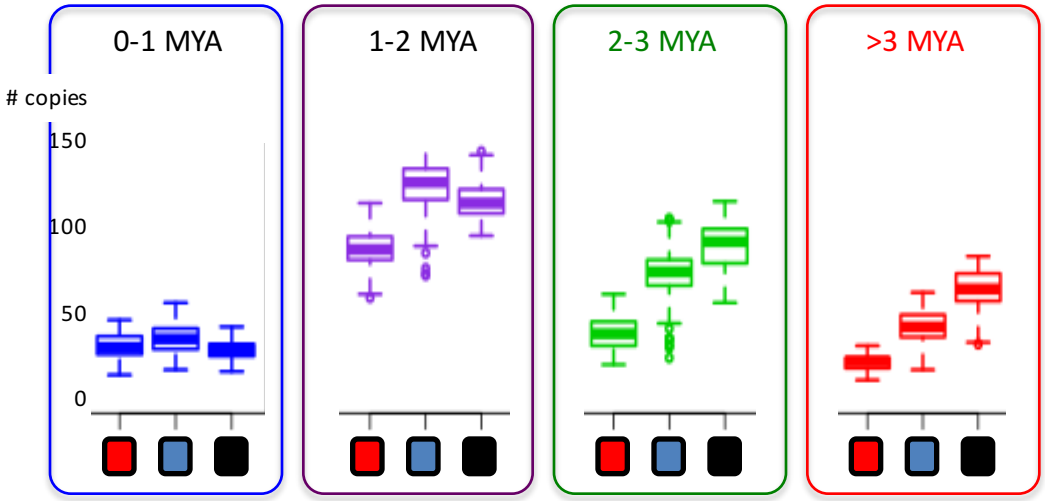


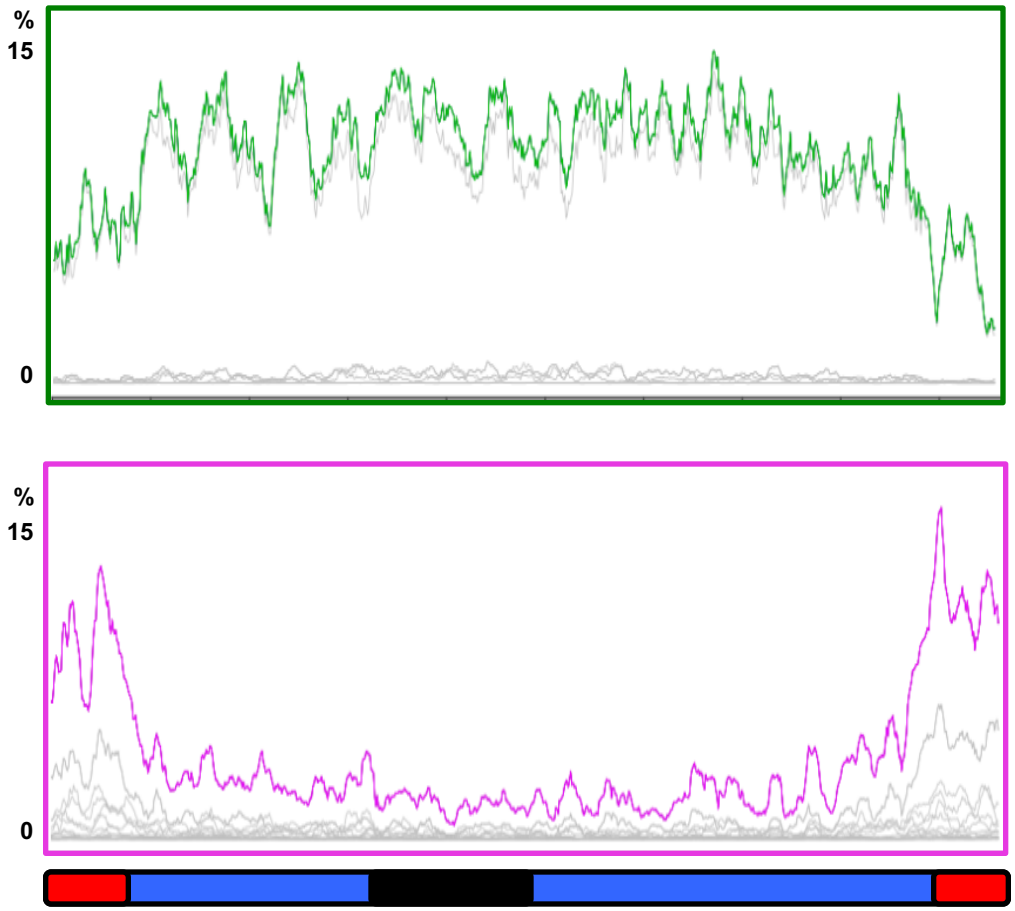
- silenced until polyploidization…
- … but shared betw A-B-D

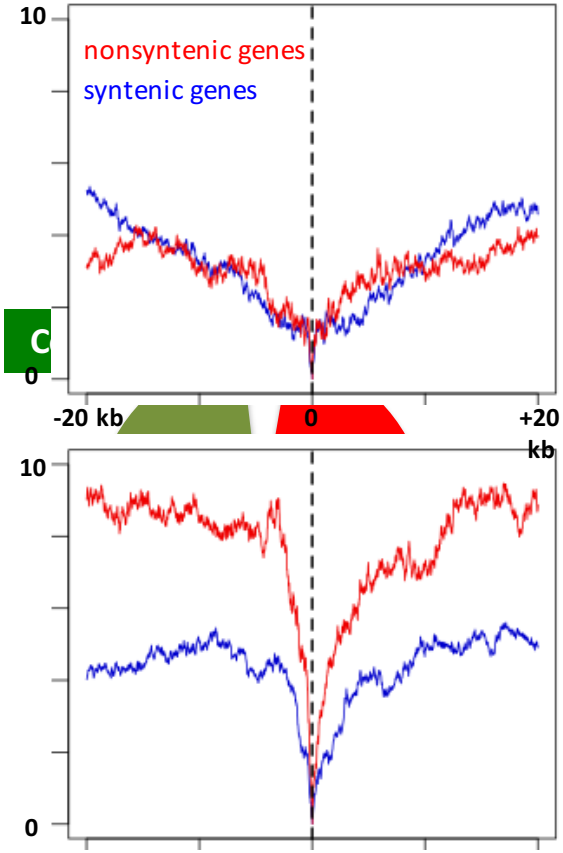


❑ Evolutionary forces driving TE distribution






%
15
0
%
15
0

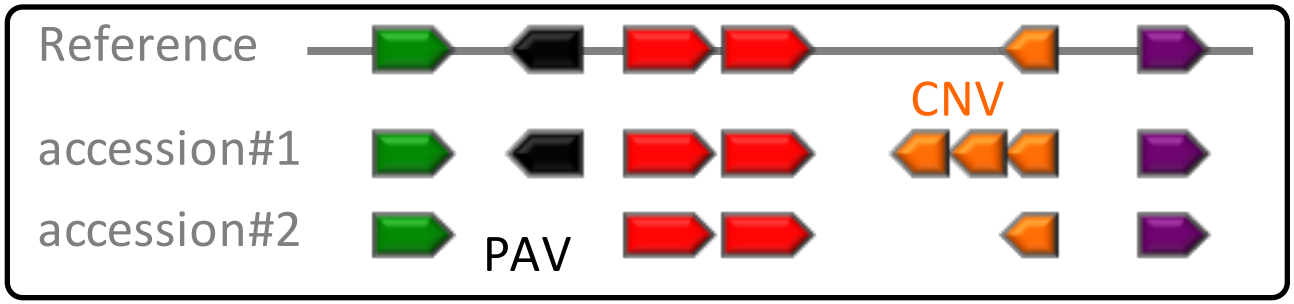

10
nonsyntenic genes
syntenic genes
C
0
-20 kb
0
+20 kb
10
0

Structural Variations

- CNVs
- PAVs

❑ Structural Variations (SVs)

- Small indels
- Inversions, translocations
- **Duplications & Deletions --> CNVs & PAVs**

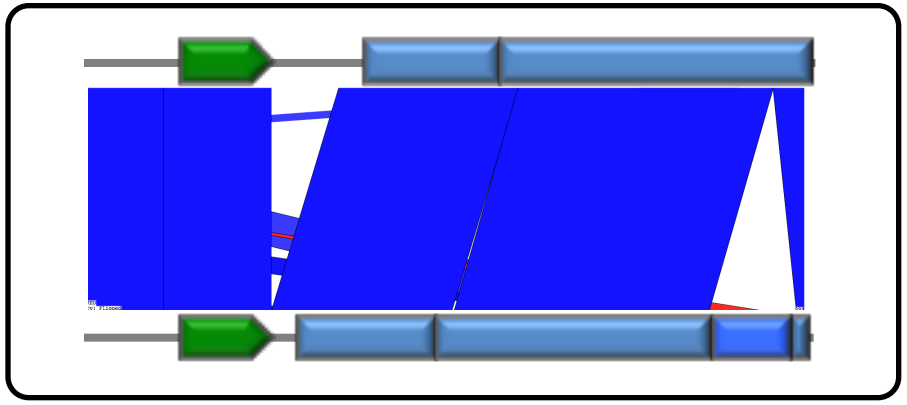


Main questions:

- Extent of SVs among the *Triticeae,* hexaploid wheat acc.?
- Impact of polyploidization?
- Relationships betw SVs and chr. organization?
- Impact on phenotypes?

❑ Structural Variations (SVs) – **Detection**

- Aligning orthologous sequenced loci

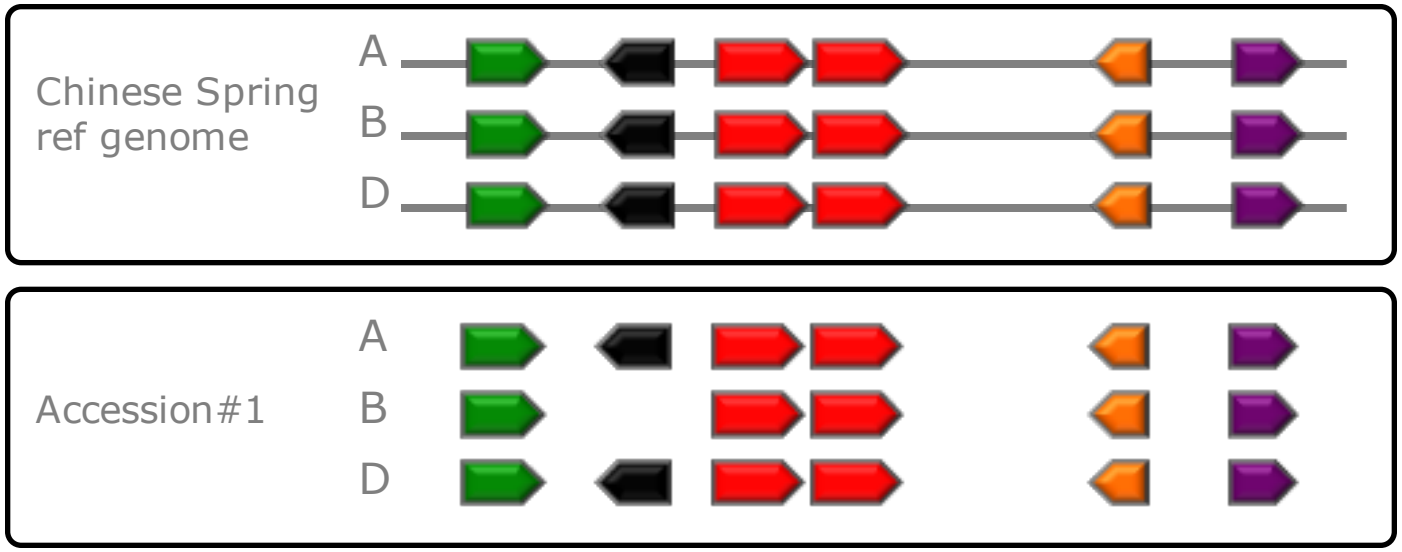


ACT view

- Using resequencing data (short read-based)
  - not properly mapped paired-reads
  - split reads
  - depth of coverage

→ ***Limitations*** *in polyploid TE-rich genomes*

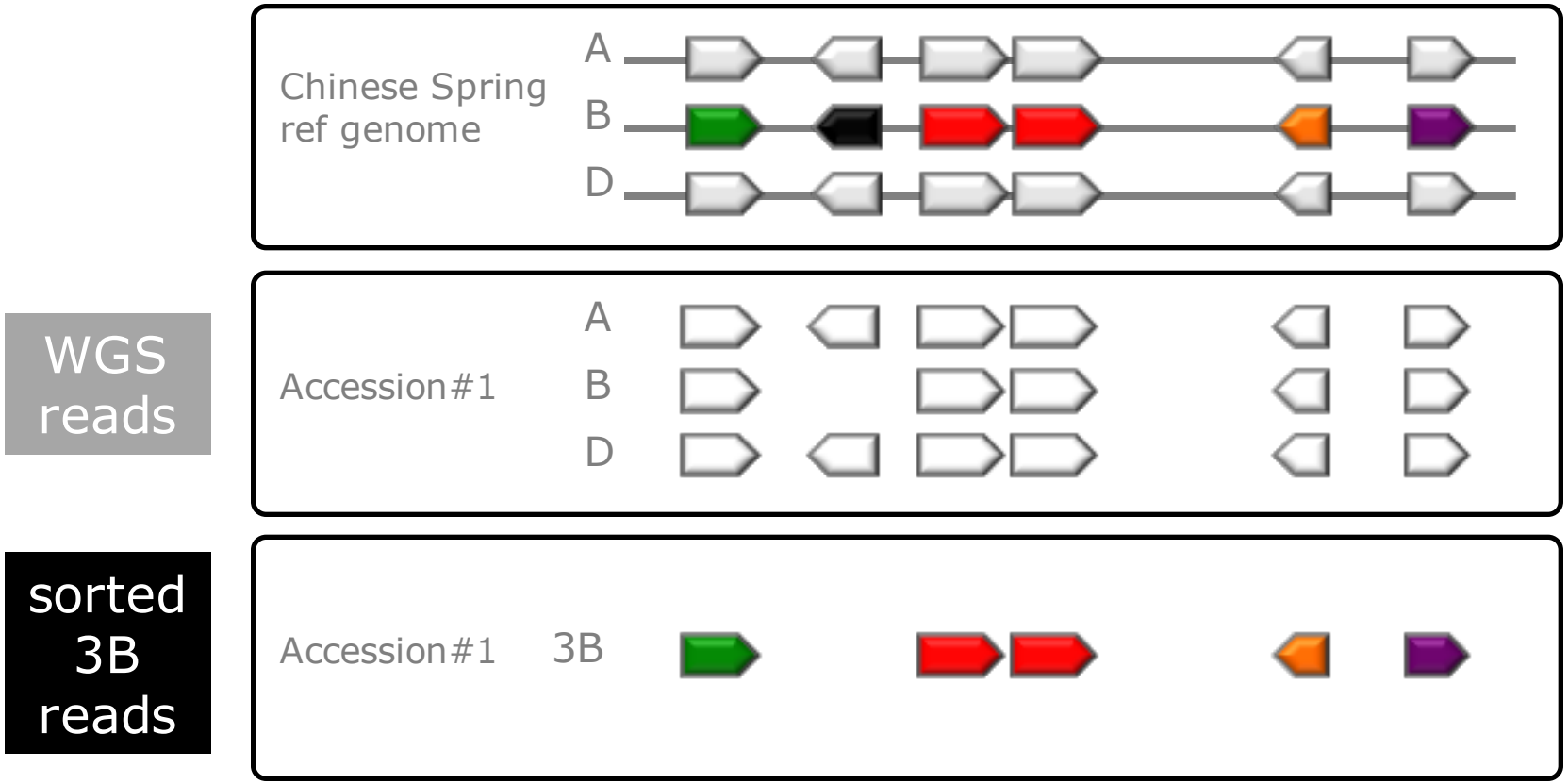
❑ Resequencing data



WGS reads

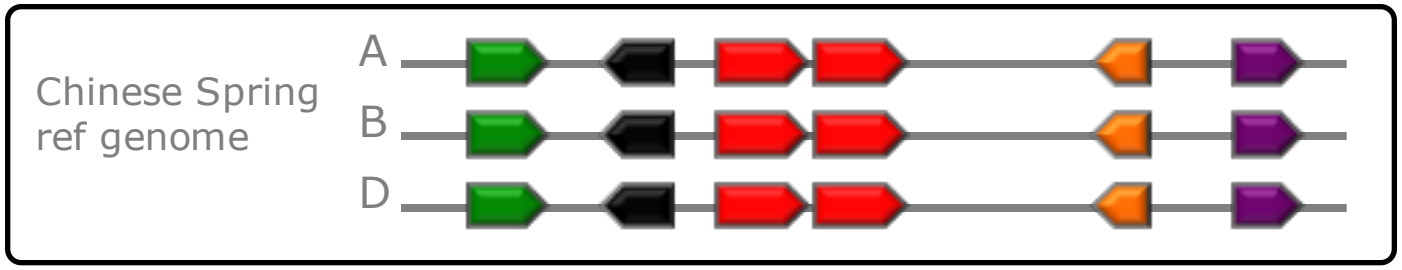
- **limitations:** homeologs and paralogs (repeated genes)

❑ Resequencing data



- **advantage:** diploid context
- **limitations:** 3B DNA amplified before seq.

❑ Resequencing data



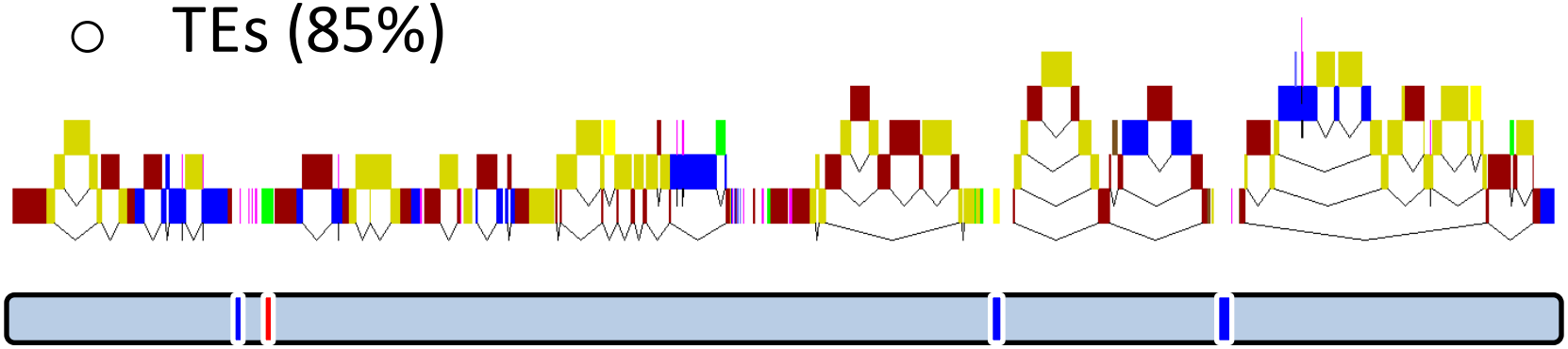
sorted 3B reads

45 accessions

22 hexaploids: *T. aestivum*
6 hexaploids: *T. macha, spelta*
17 tetraploids: *T. durum, dicoccoides, dicoccum, carthlicum*

- Illumina 2x100bp
- Depth: **~40x**

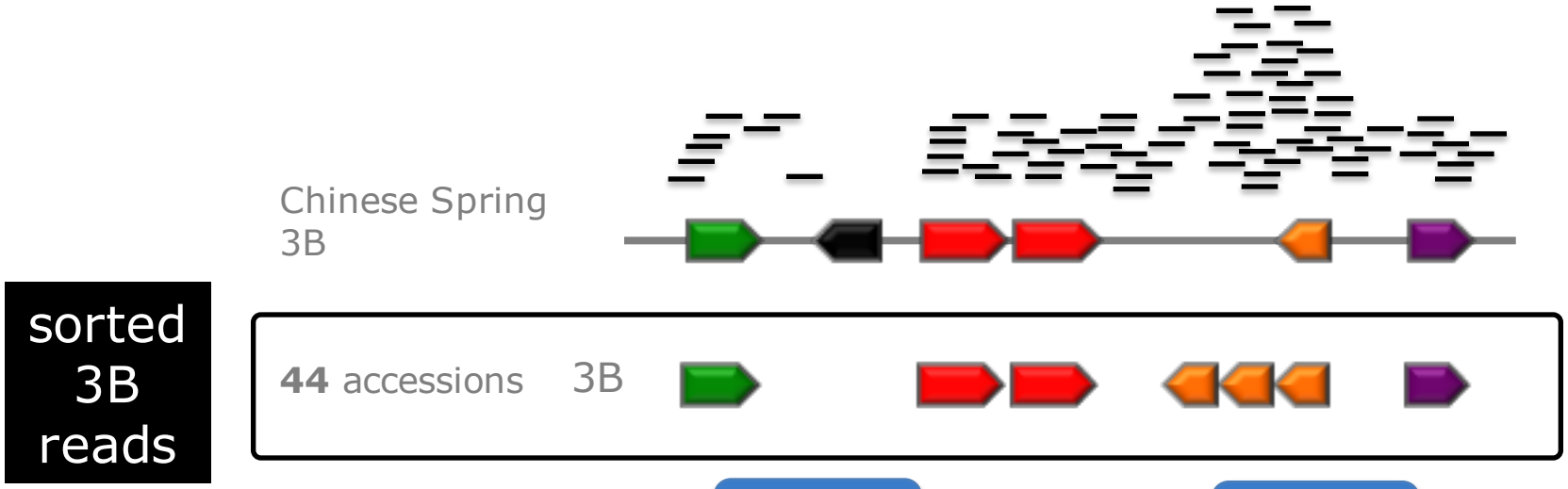
- TEs (85%)



- Genes (2%)

❑ SVs in genes – Methodology

- BWA & samtools



PAVs

<**10%** of the gene length covered by reads

CNVs

depth of coverage-based approach

Normalization:

- GC%
- sequencing depth
- gene length
- $\log_2(cov^{acc}/cov^{Chinese\ Sp})$

❑ SVs in genes – Results

PAVs

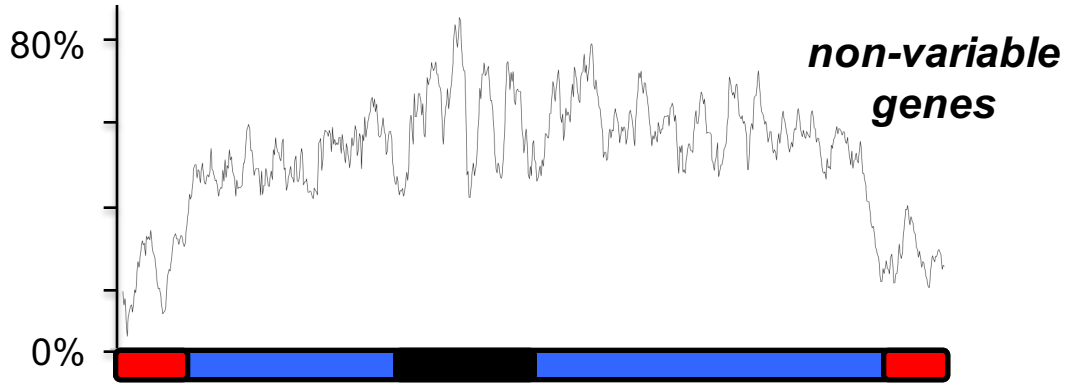
XX% genes deleted in 1+ accessions [XX..XX]

CNVs

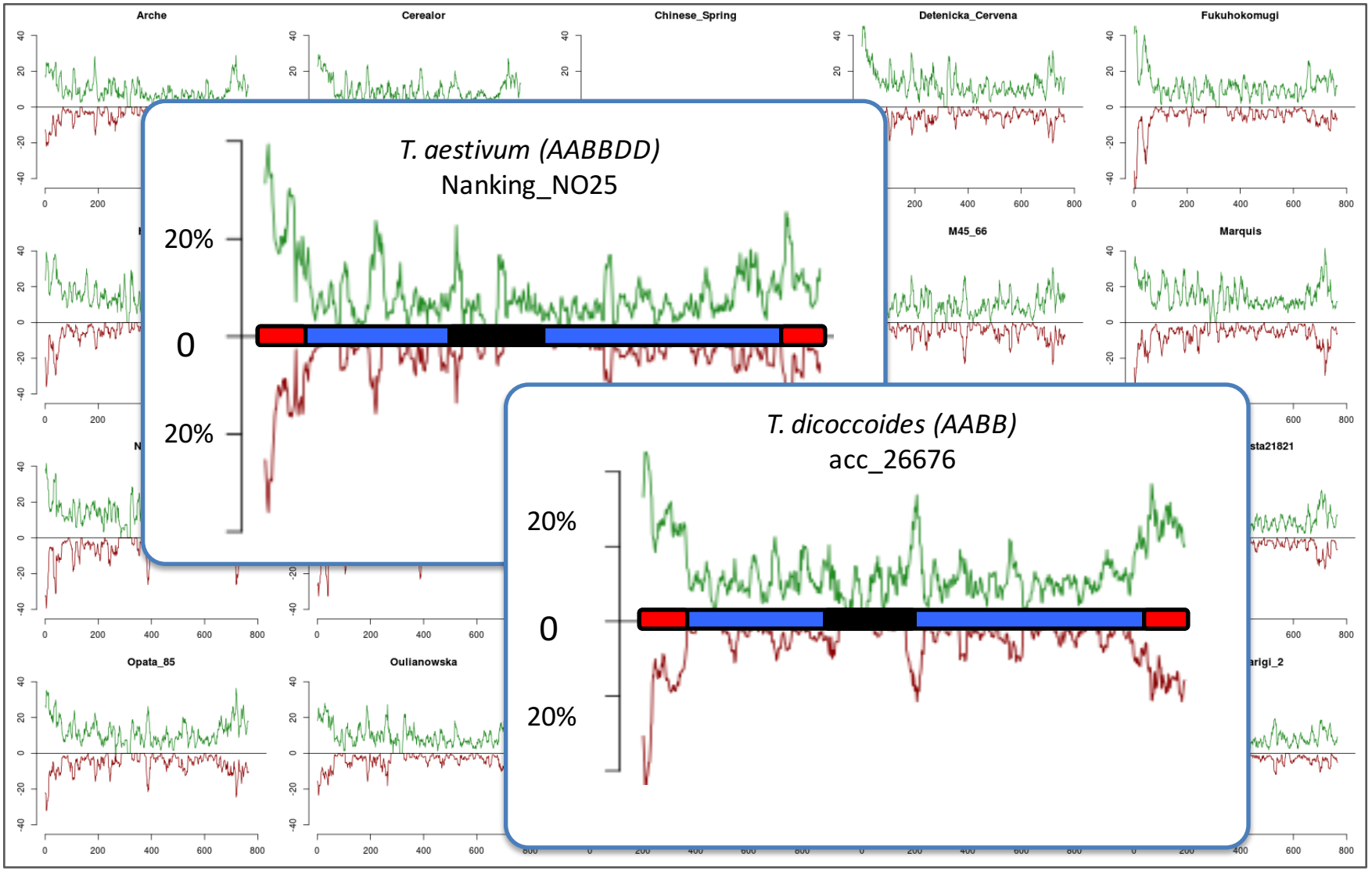
down^{CNVs}: X% of the 3B genes (on average per acc.)

up^{CNVs}: X% "

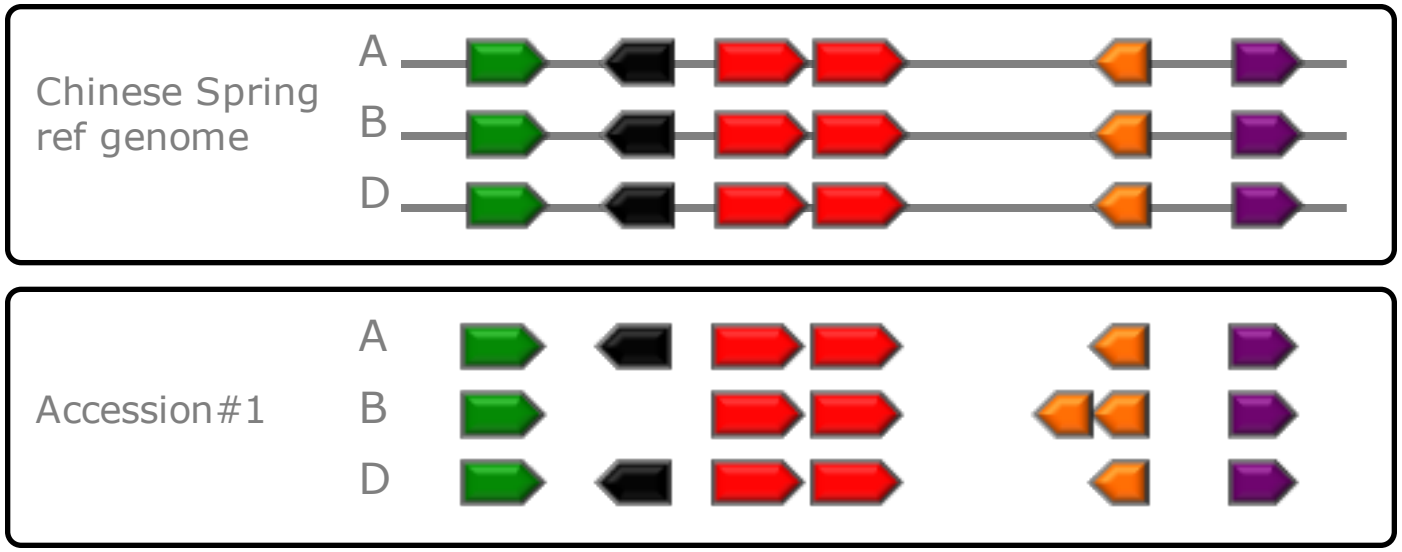
XXXX (**XX%**) genes with no variation among 45 accessions



❑ SVs in genes – Results



❑ SVs in genes – Methodology

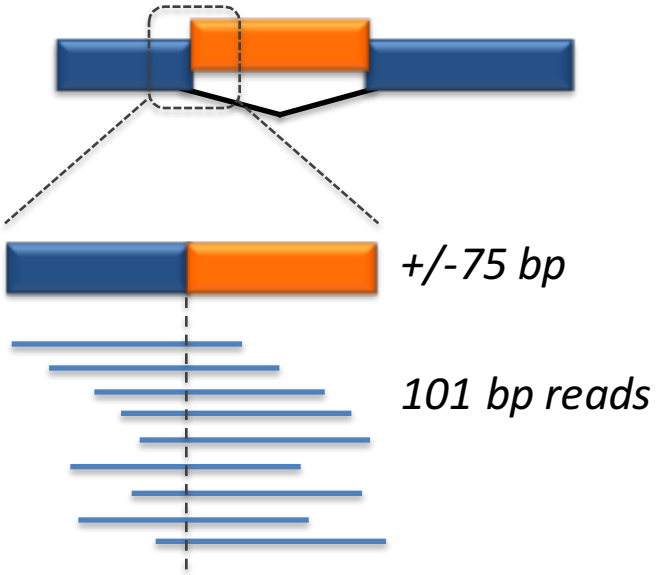


WGS reads

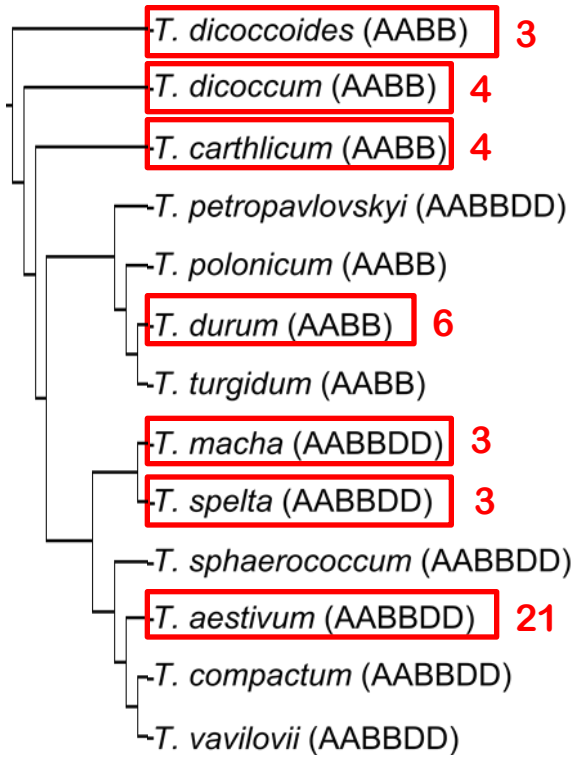
=> develop a fine-tuned strategy?... in progress

❑ SVs in TEs – Methodology

TE junction-based approach (**PAVs** only)



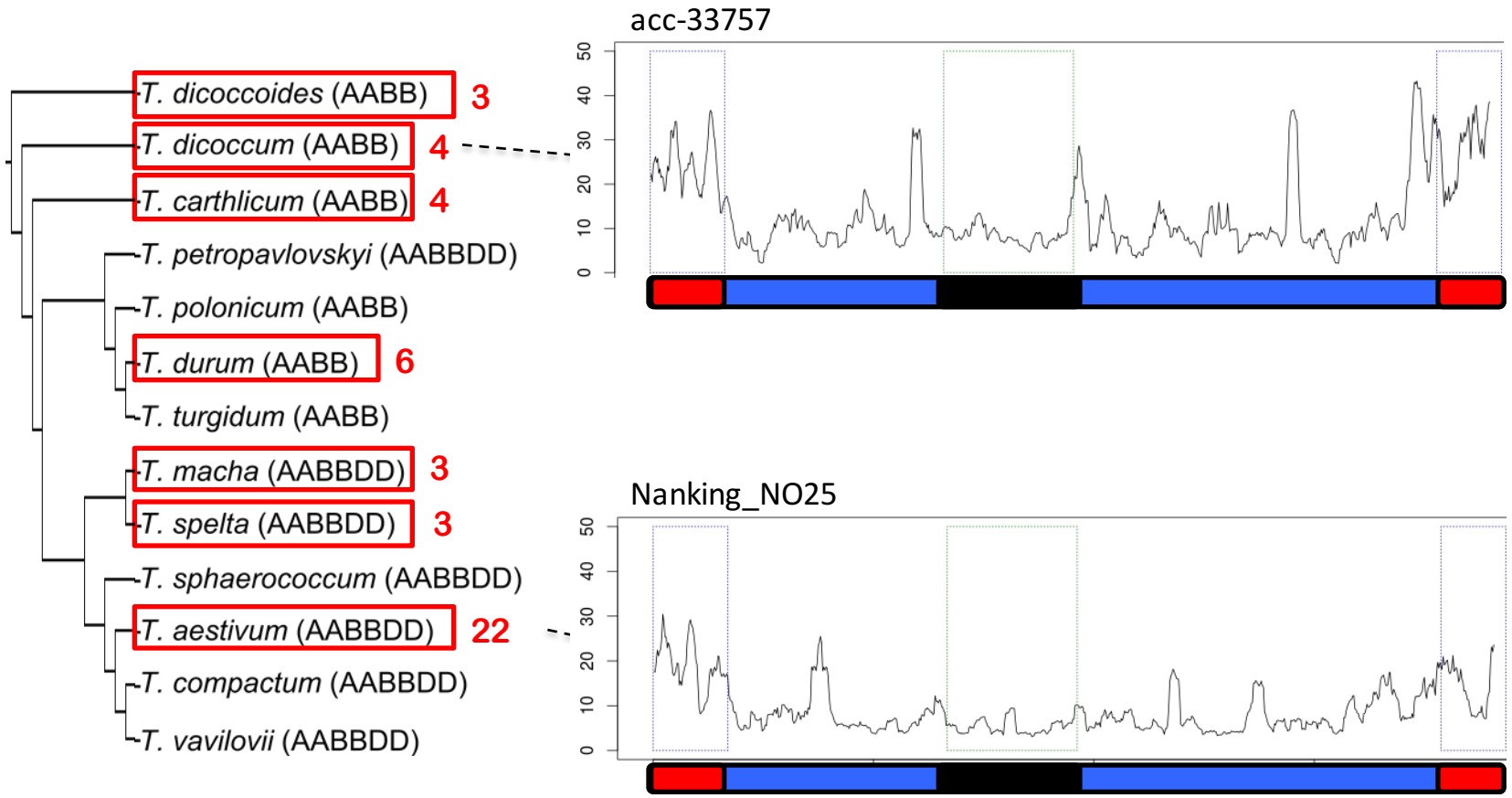
❑ SVs in TEs – Results



3B ➔ ~**500k** loci to study TE-PAVs along chr3B

- Polymorphic loci among 45 accessions: XX%
- Average per accession: XX% [XX% .. XX%]

❑ SVs in TEs – Results



❑ Conclusions

- Gene PAVs limited (on 3B)
- CNVs: **XX%** of the 3B genes (versus ~10% in barley *[Munoz-Amatriain et al. 2013]* 14 wild+cultiv. genotypes)
- High level of **TE**-related SVs
- Importance of **chr. extremities** in the diversity of *Triticeae* (also observed in barley)

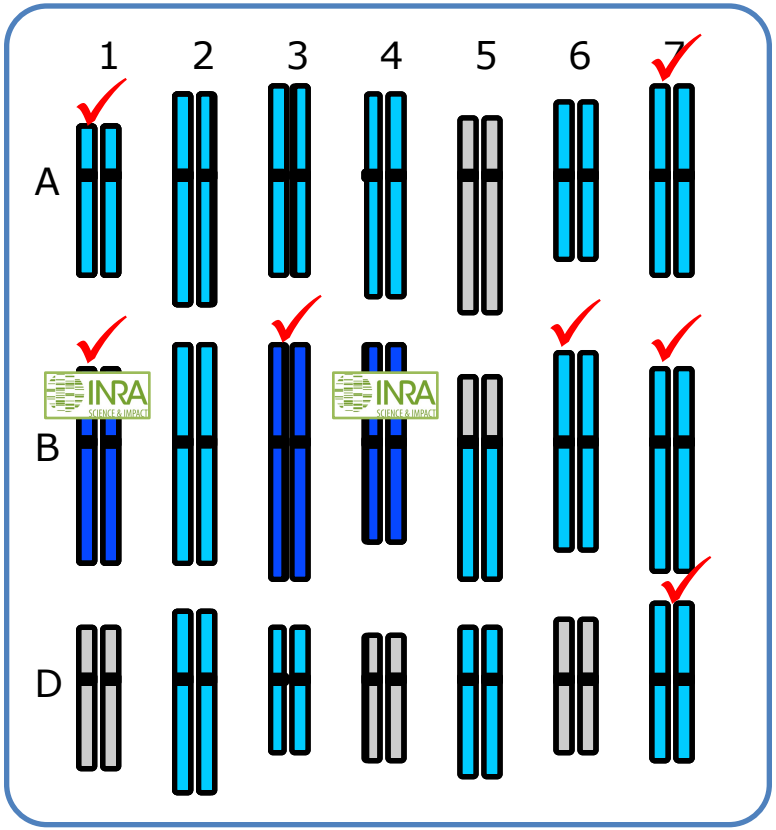
❑ Next

- Validate the approach/thresholds used for CNV calling
- SVs at the whole genome level
  - ->use 3B-*vs*-3B results as QC
  - ->increase sample size through exon capture
- GO term enrichment
- Relationships betw TE SVs / gene expression
- Unmapped reads (pangenome)

Wheat genome seq initiatives in 2016:

- MTPseq 1A, 1B, 6B, 7A, 7B, 7D
- **IWGSC Whole Genome Assembly (NRGene)**
- TGAC WGS (several varieties)
- U Maryland WGS Pacbio+Ill
- UC Davis *Ae. tauschii* WGS+MTPseq
- BGI *T. urartu* WGS+MTPseq
- Wild Emmer Wheat (NRGene)

chr-by-chr approach, status in 2015…



IWGSC-Whole Genome Assembly

Partners:

N. Stein

C. Pozniak

J. Poland

A. Diestelfeld

A. Scharpe

G. Ronen

M. Thompson

K. Eversole, J. Rogers

F. Choulet

Strategy:

WGS Illumina 180x - 3 MP lib

DeNovoMAGIC™ 2.0

Timeline:

- Aug 2015 -> start
- Sept
- Oct
- Nov -> Sequencing done
- Dec -> Assembly v0.1

  QC *(M. Mascher, F. Choulet)*
- Jan. 2016 -> Assembly v0.2

IWGSC-Whole Genome Assembly

				N50	
2013	T.ur	BGI	T.ura-BGI	64 kb /	19000 scaff
2013	Ae.t	BGI	Ae.ta-BGI	58 kb /	19000 scaff
2014	CS	IWGSC	CSS	2 kb /	>1M scaff
2014	CS(3B)	GDEC/CNS	3B-pseudo	892 kb /	296 scaff
2015	Synth	IPK/JGI	Syn-JGI	21 kb /	120000 scaff
2015-Jul	WEW	NRGene	WEW-NRGene	7000 kb /	414 scaff
2015-Dec	CS	IWGSC	IWGSC-WGA	**7394 kb /**	**547 scaff**

IWGSC-Whole Genome Assembly

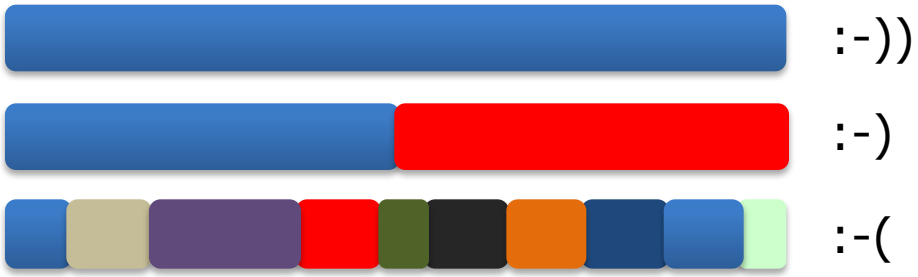
QC :: Completeness?

- exons: **98.7%** match with 100%id-100%ov
- ISBPs (4.2M): **97.7%** match with 100%id-100%ov
- WGPtags-4B (0.9M): **96.2%** match with 100%id-100%ov

➔ Completeness+++

IWGSC-Whole Genome Assembly

QC :: Chimeras?



- alignment to CSS genes and ISBPs
- alignment to MTPseq (3B, 1B)
- alignment to genetic map (POPseq, CsRe)
- alignment to physical map (WGPtags)
- alignment to HiC map

➔ Chimeric scaffolds found (<200) -> corrected in v0.2/v0.3

IWGSC-Whole Genome Assembly

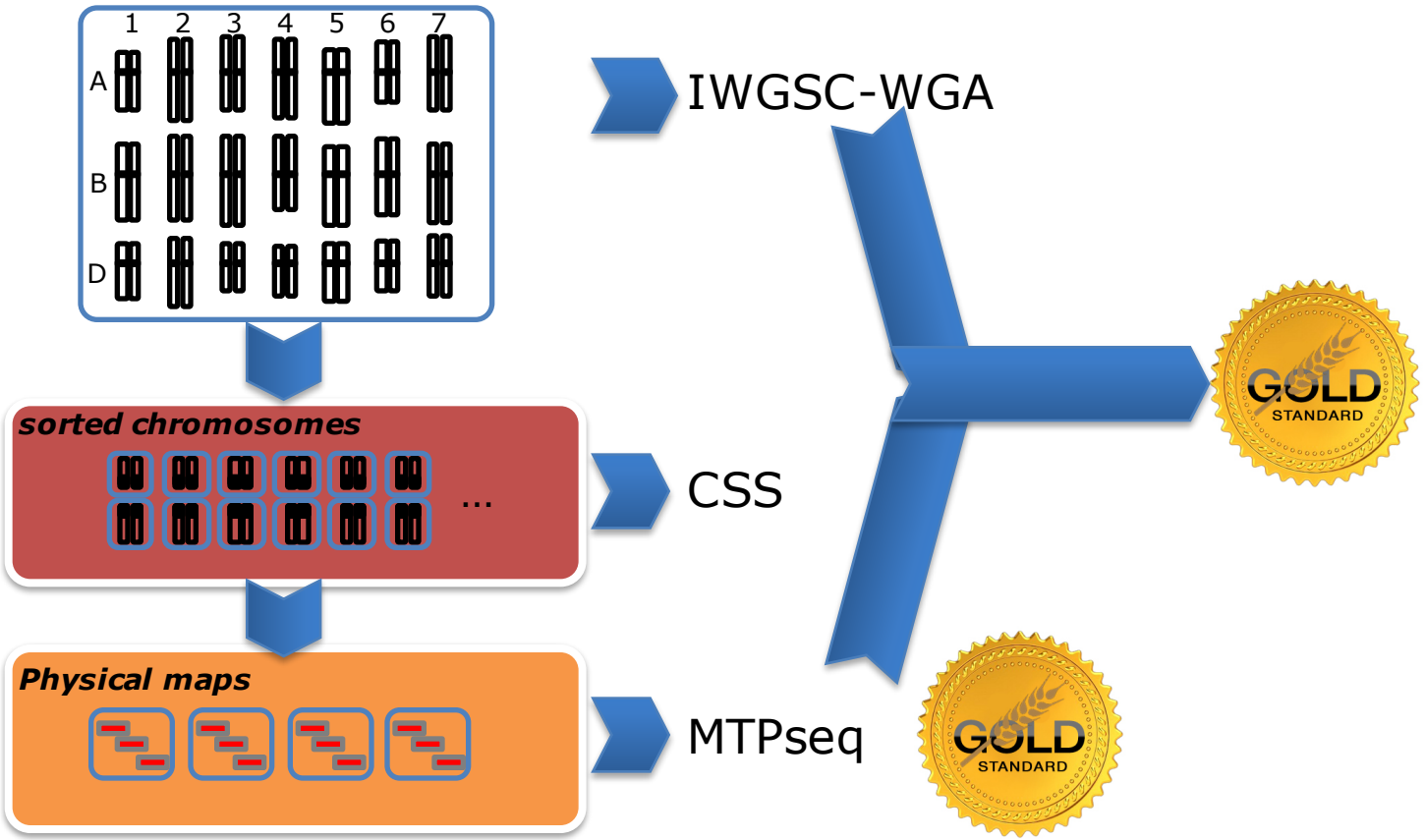
IWGSC-WGA v0.2 metrics:

#Scaff≥2kb:	37,872
Size:	14.532 Gb
Gaps:	1.8%
L50:	7.058 Mb / 566 scaff
L90:	1.261 Mb / 2,363 scaff
max:	45.794 Mb

21 pseudomolecules constructed with HiC data (IPK)

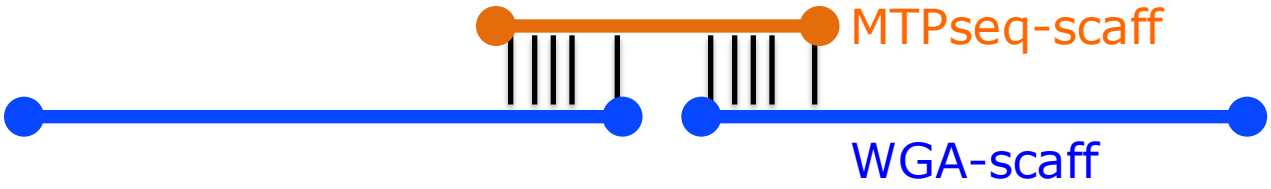
→ 14.0 Gb (**96%**) IWGSC-WGA ordered

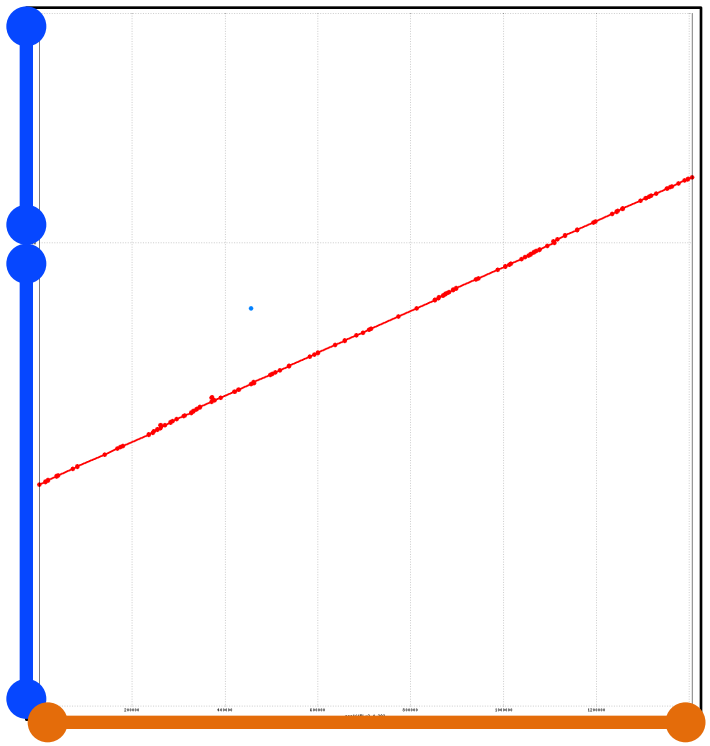
IWGSC roadmap update



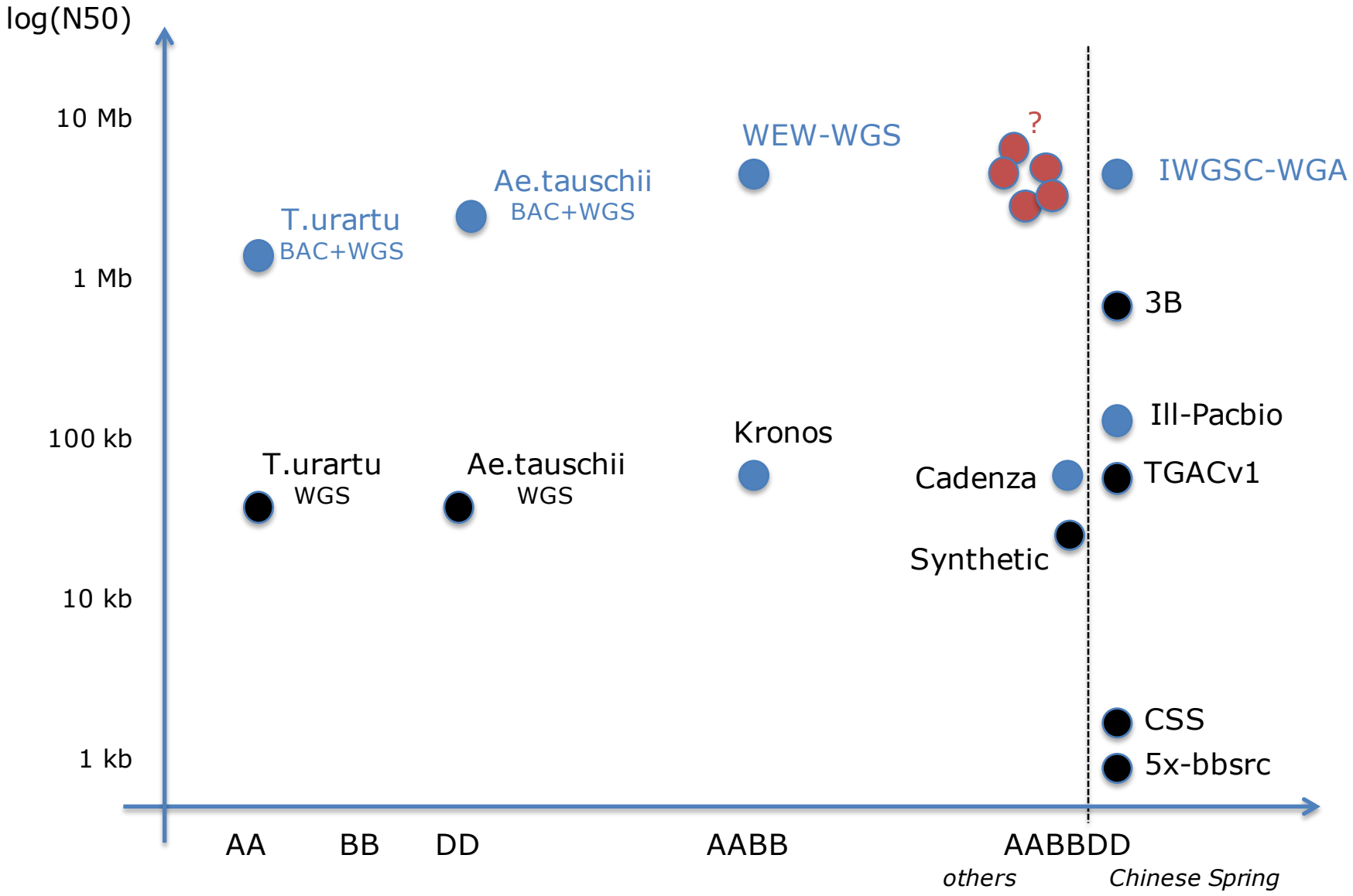
- Integration WGA ⇔ MTPseq in progress…

Strategy based on comparing ISBPs (speed++ specificity++)





207 WGA-scaff joined by 1B-MTP-scaff
representing 593 Mb


log(N50)
10 Mb
1 Mb
100 kb
10 kb
1 kb
T.urartu
BAC+WGS
Ae.tauschii
BAC+WGS
WEW-WGS
?
IWGSC-WGA
3B
Ill-Pacbio
TGACv1
CSS
5x-bbsrc
T.urartu
WGS
Ae.tauschii
WGS
Kronos
Cadenza
Synthetic
AA
BB
DD
AABB
AABBDD
others
Chinese Spring

Thanks

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