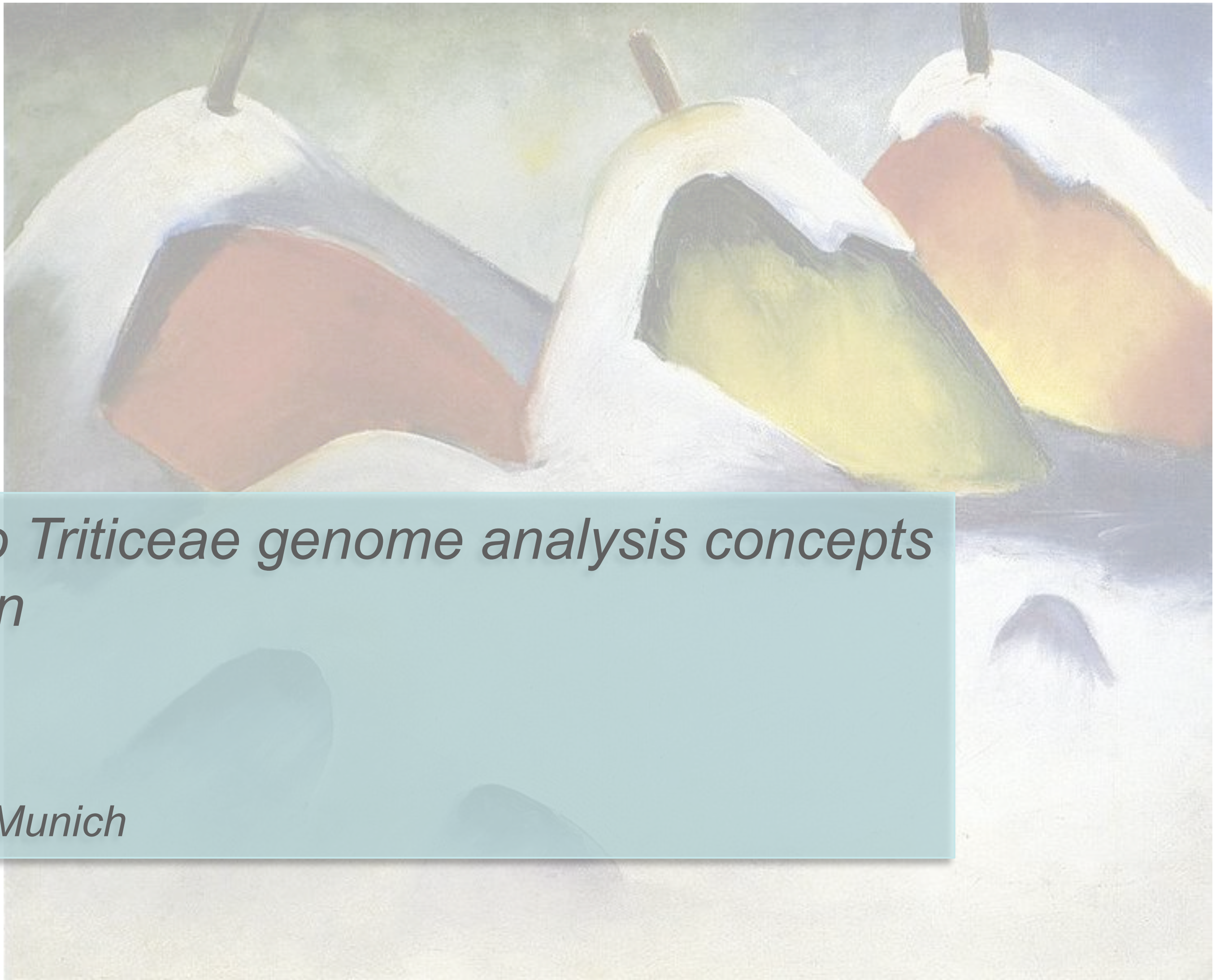
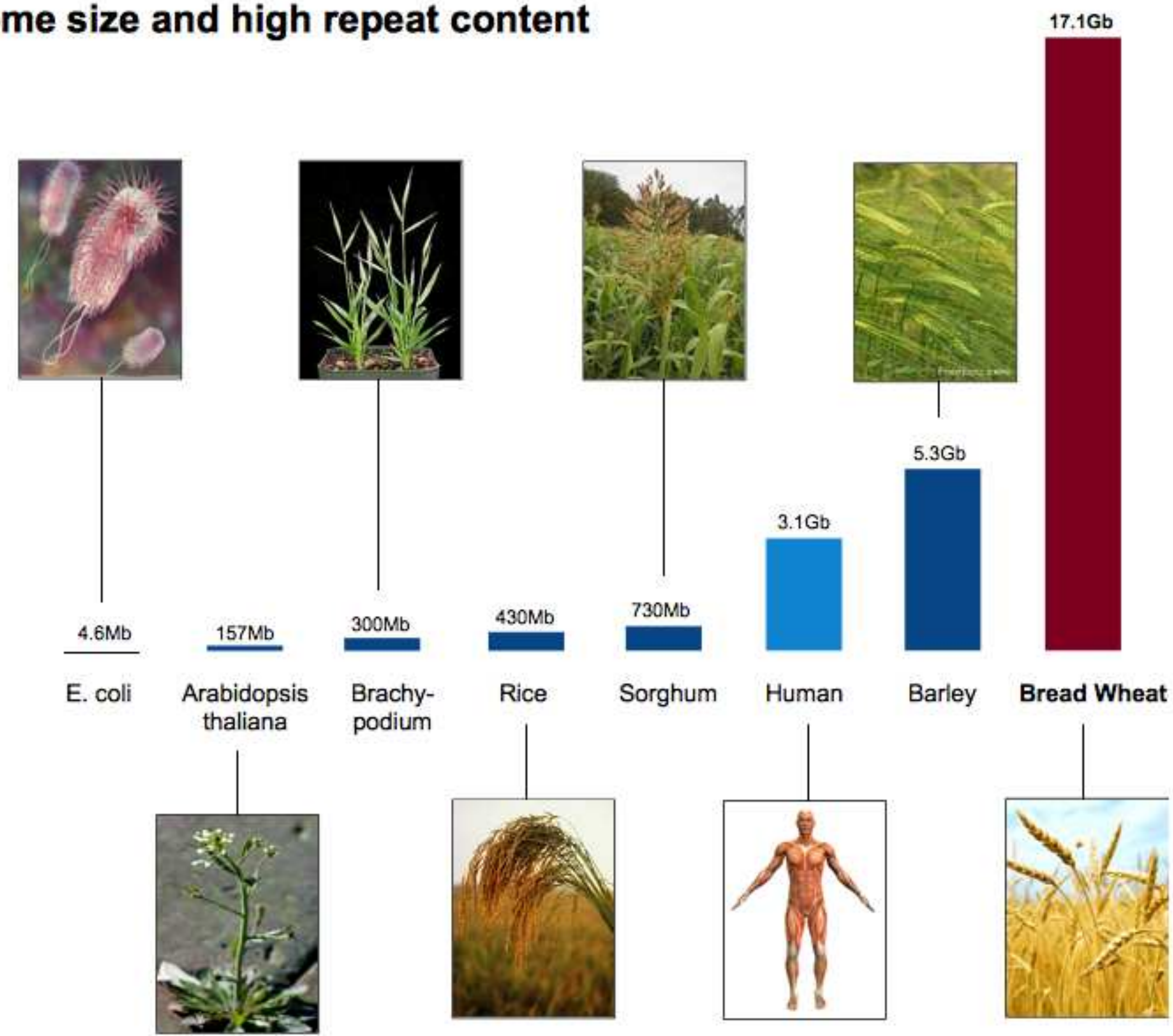


An Introduction into Triticeae genome analysis concepts and data generation

Klaus Mayer
MIPS, Helmholtz Center Munich

The Challenge

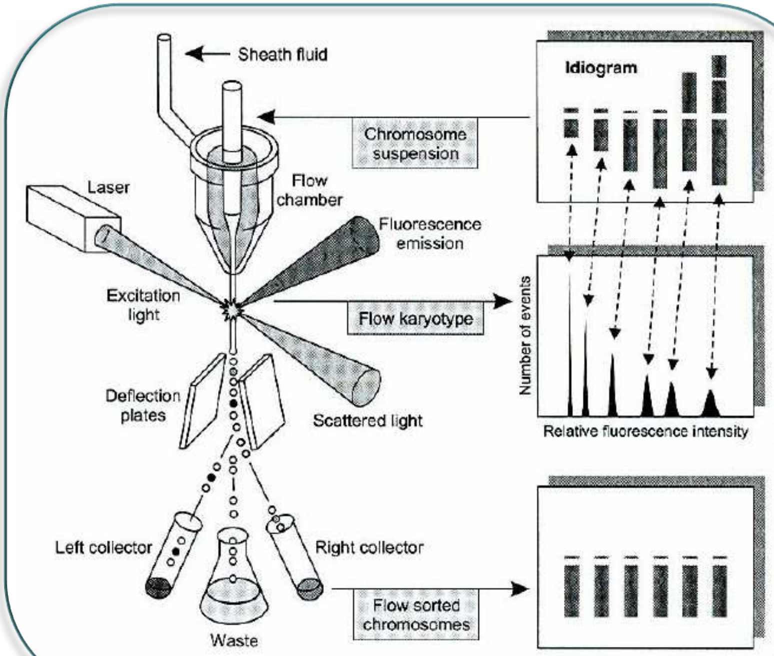
- ► **Big genome size and high repeat content**

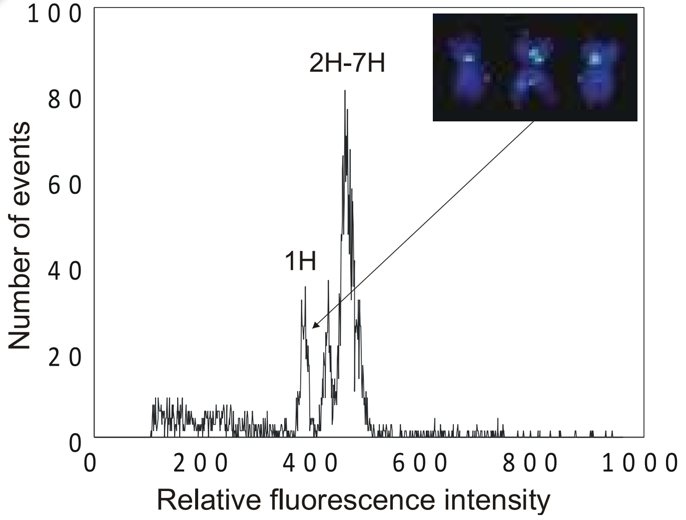


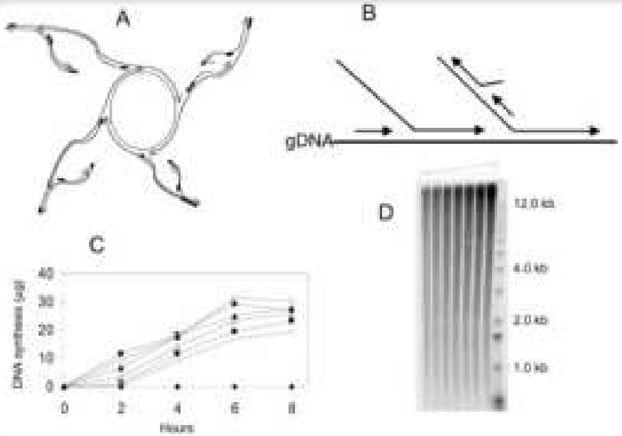
Outline

- Barley: genome zipping and genome stratification puts the pieces together
- Accessing wheat by WGS using „*in silico* exon capture“
- Rye, a highly rearranged *Triticeae* genome unzipped
- Rye B‘s: the path of the aliens

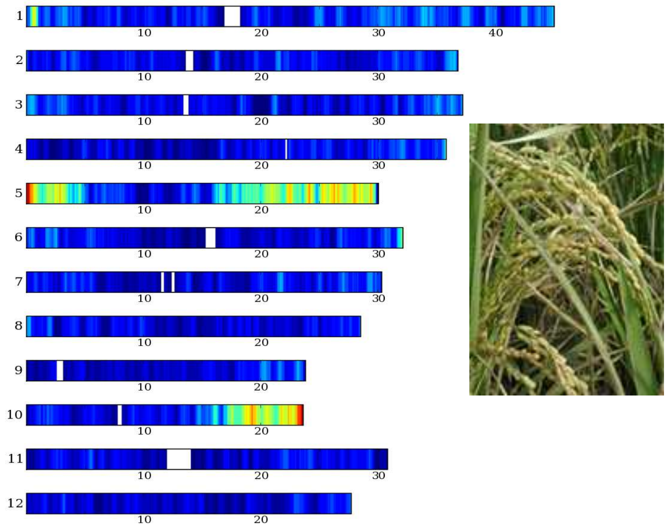
Reduction of complexity by chromosome sorting

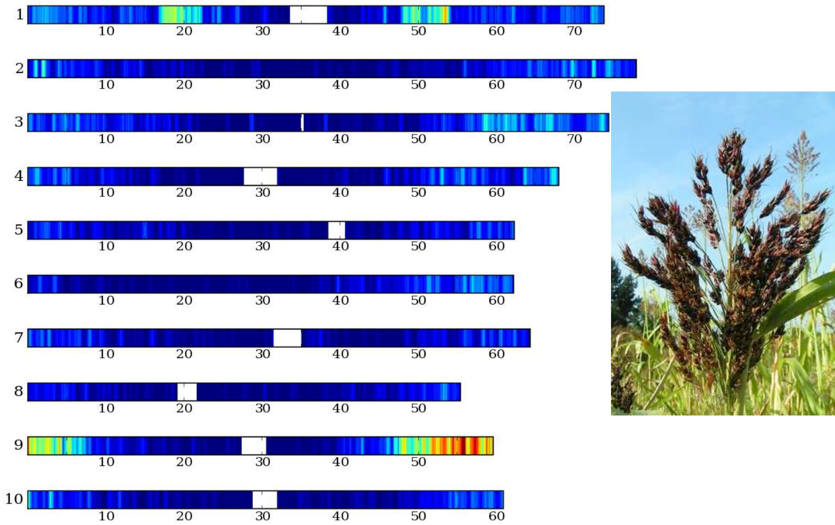


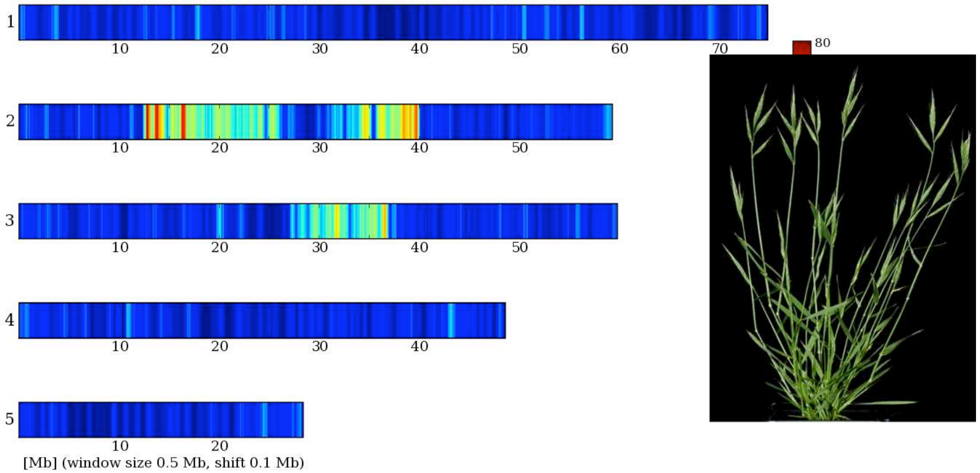




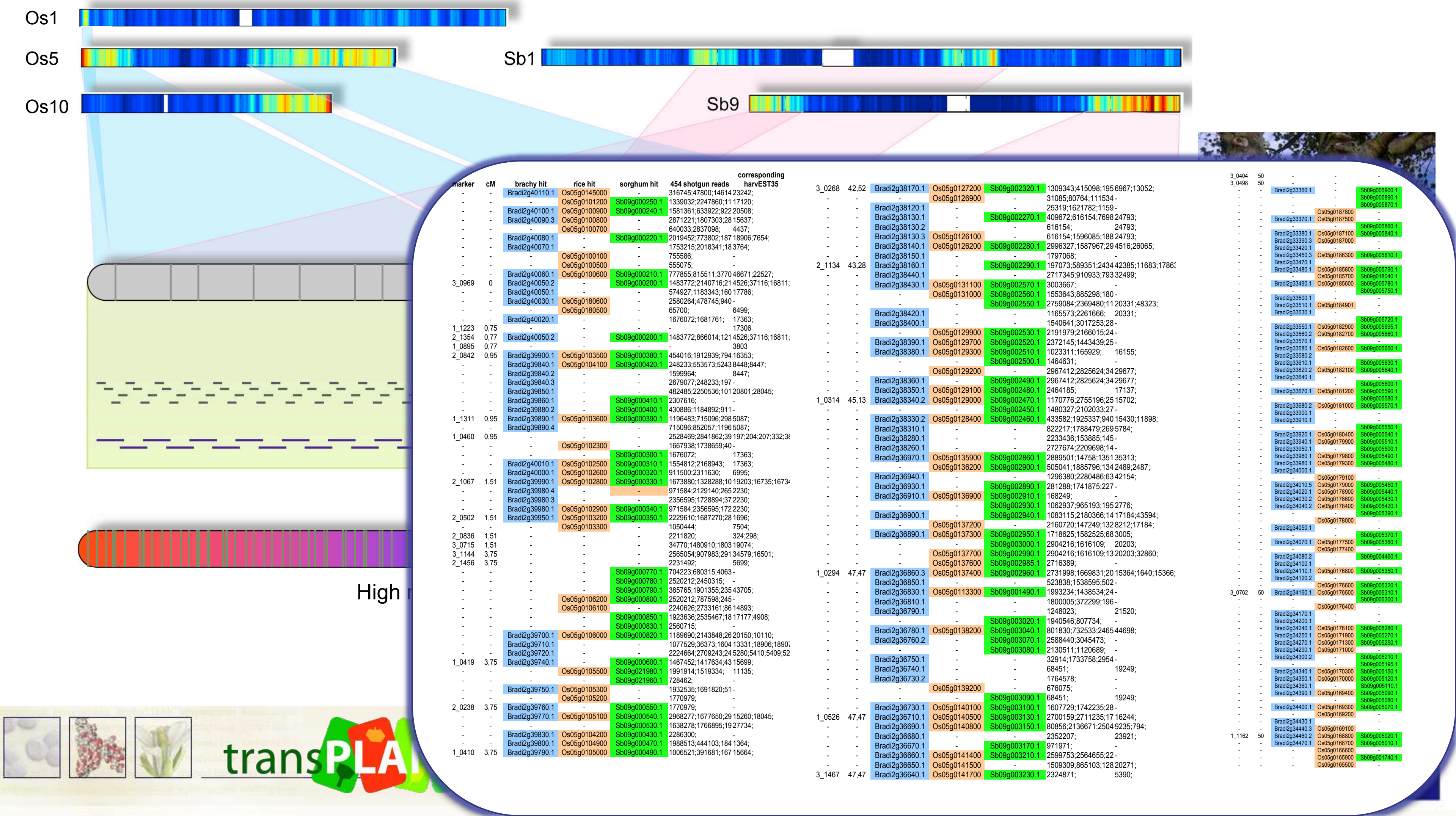
Barley vs *Brachypodium*, *Sorghum* and rice
-Synteny on a per gene resolution-







Syntenic Integration generates a „GenomeZipper“



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-	-	Bradi2g40070.1	-	-	1753215;2018341;18	3764;
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-	-	Bradi2g39840.2	-	-	1599964;	8447;
-	-	Bradi2g39840.3	-	-	2679077;248233;197	-
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-	-	Bradi2g39860.1	-	Sb09g000410.1	2307616;	-
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-	-	-	-	Sb09g000300.1	1676072;	17363;
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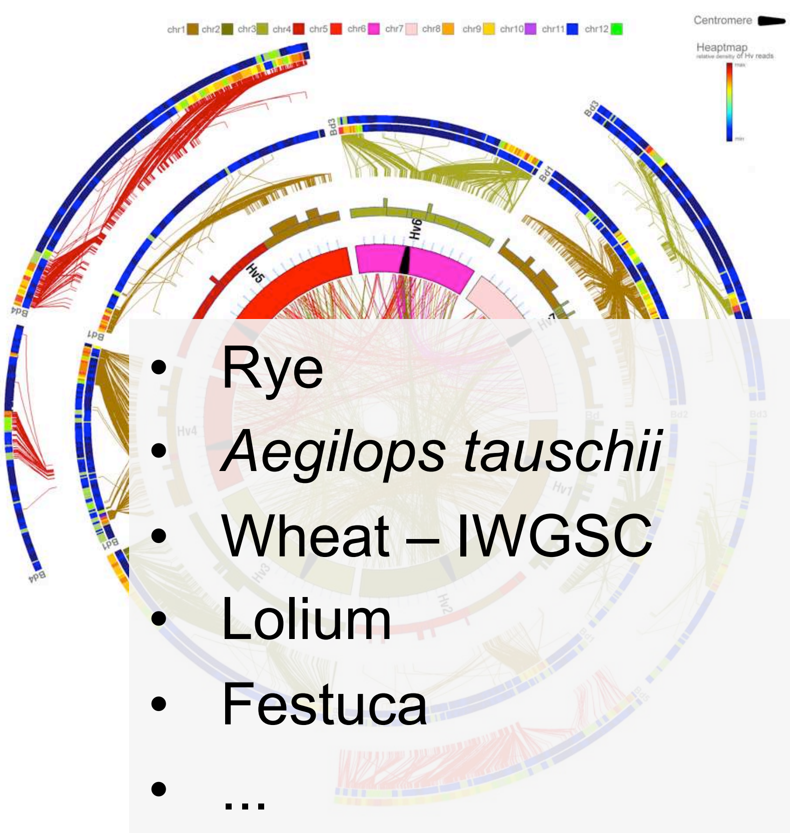
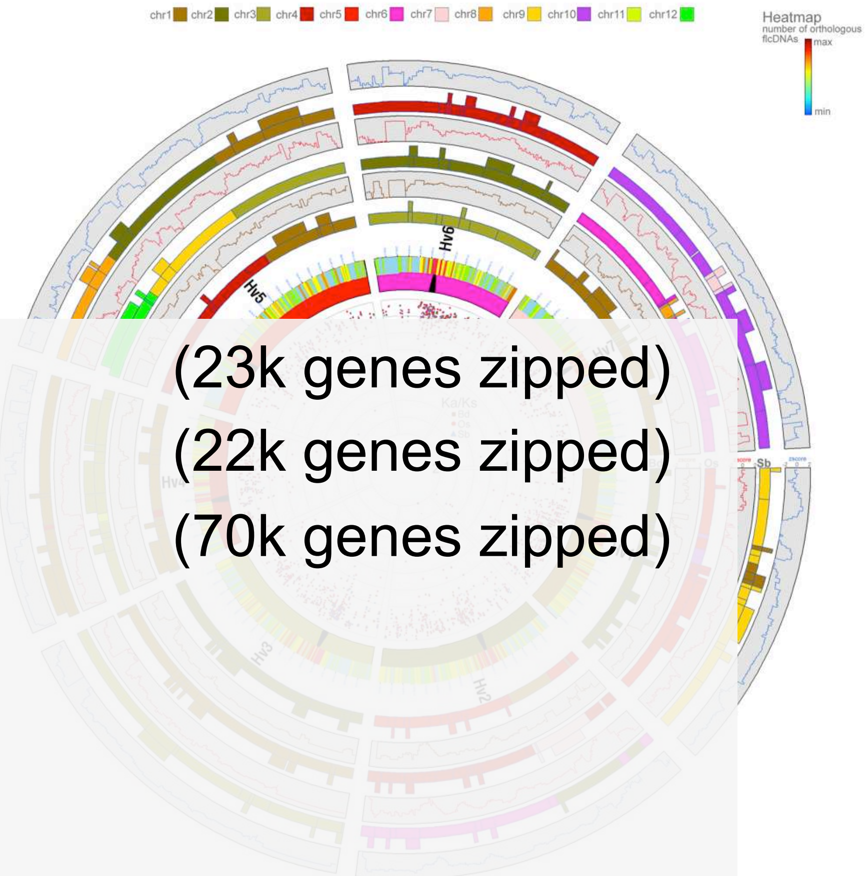
A powerful shortcut towards an ordered gene map of the huge *Triticeae* genomes





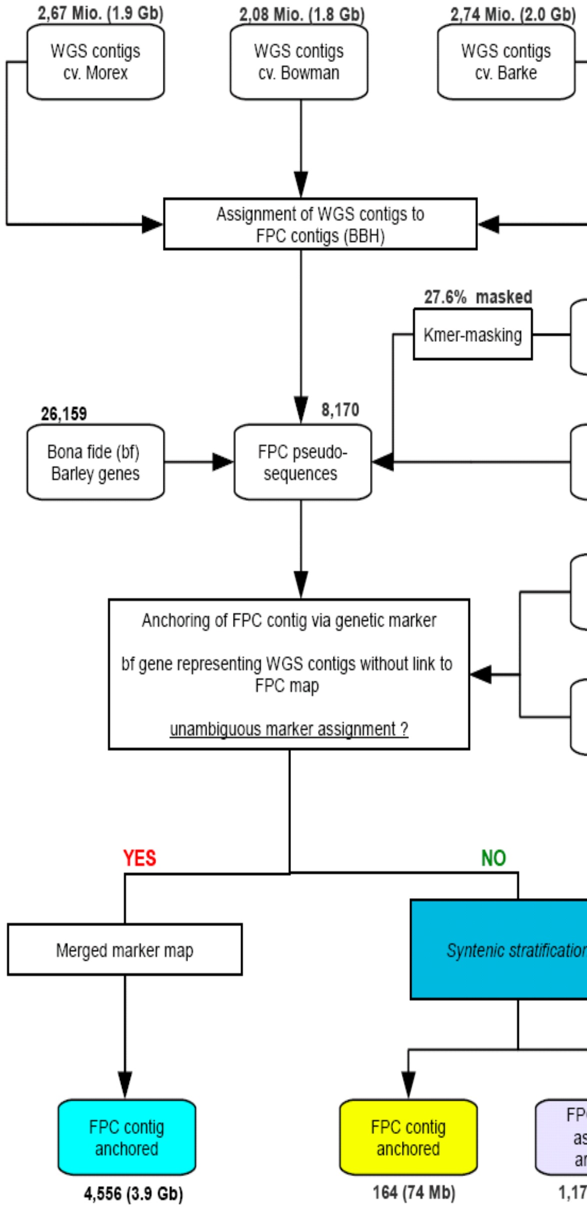
- Rye (23k genes zipped)
- *Aegilops tauschii* (22k genes zipped)
- Wheat – IWGSC (70k genes zipped)
- Lolium
- Festuca
- ...

Genome stratification in barley

Combined genetic and physical map
build scaffold

+ Sequence enriched via 80x WholeGenomeShotgun
6,200 BACs,
570,000 BacEndSeqs
500,000 genetic markers
250 Gb RNA Seq

=>3,9Gb (76%) anchored
+ 650 Mb (13%) chr. arm associated



2,67 Mio. (1.9 Gb)
WGS contigs cv. Morex

2,08 Mio. (1.8 Gb)
WGS contigs cv. Bowman

2,74 Mio. (2.0 Gb)
WGS contigs cv. Barke

Assignment of WGS contigs to FPC contigs (BBH)

27.6% masked
Kmer-masking

26,159
Bona fide (bf) Barley genes

8,170
FPC pseudo-sequences

Anchoring of FPC contig via genetic marker

bf gene representing WGS contigs without link to FPC map

unambiguous marker assignment ?

YES

NO

Merged marker map

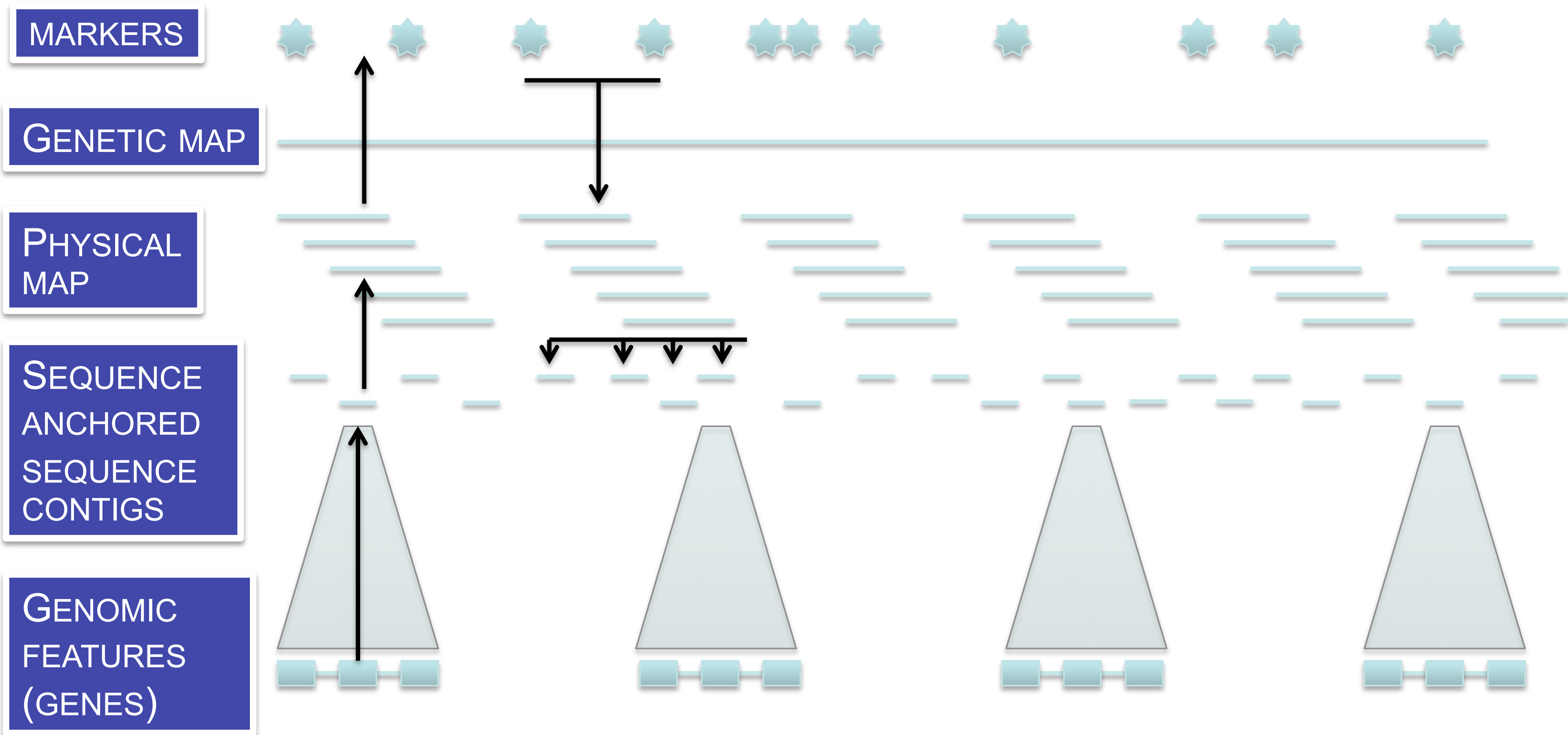
Syntenic stratificatio

FPC contig anchored
4,556 (3.9 Gb)

FPC contig anchored
164 (74 Mb)

FP
as
ar
1,17

Different hierarchies of feature connection and different layers to start the navigation



MARKERS

GENETIC MAP

PHYSICAL MAP

SEQUENCE ANCHORED SEQUENCE CONTIGS

GENOMIC FEATURES (GENES)

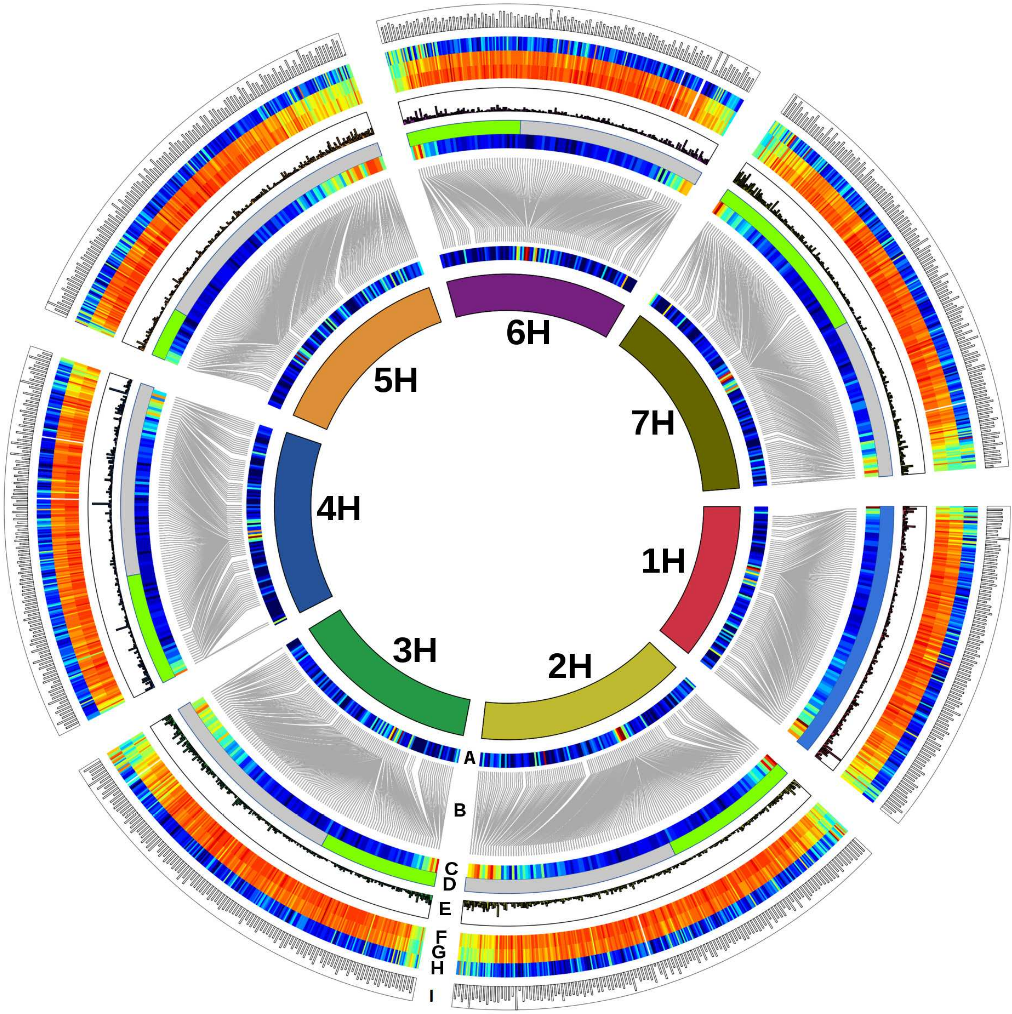
Genome stratification in barley cont.

The Barley **Gene-ome**:

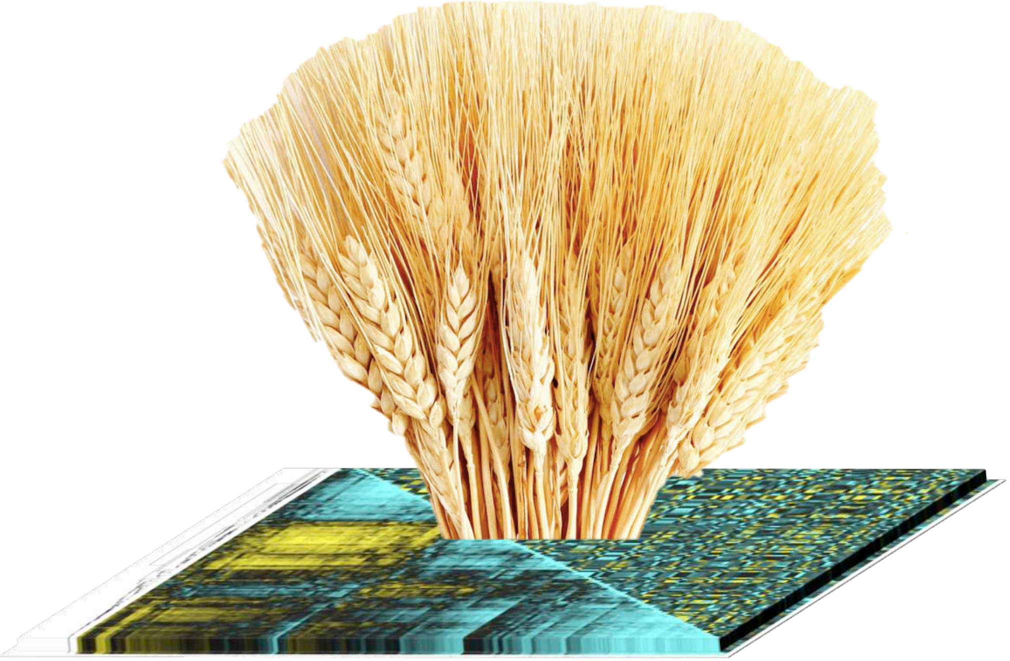
A **physical**, **genetic** and

functional sequence

assembly

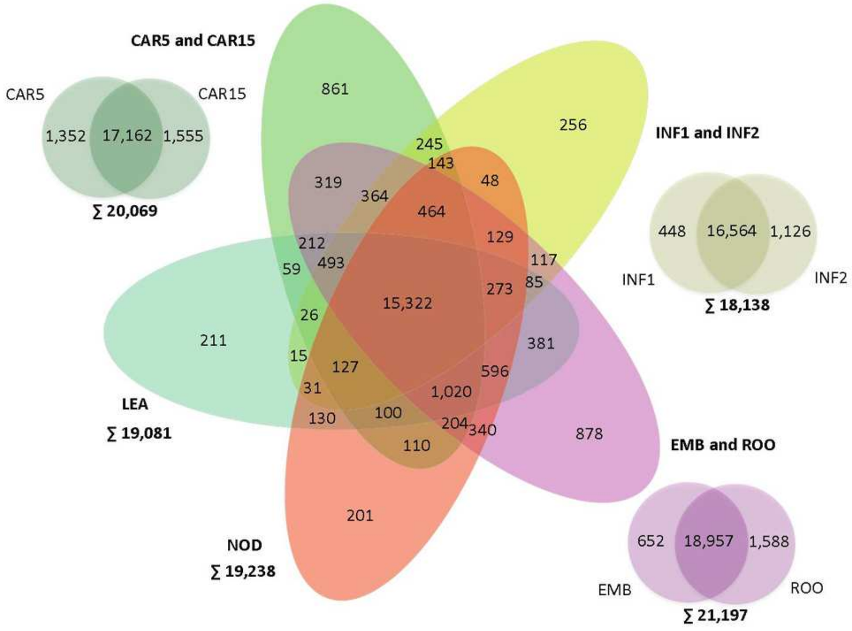


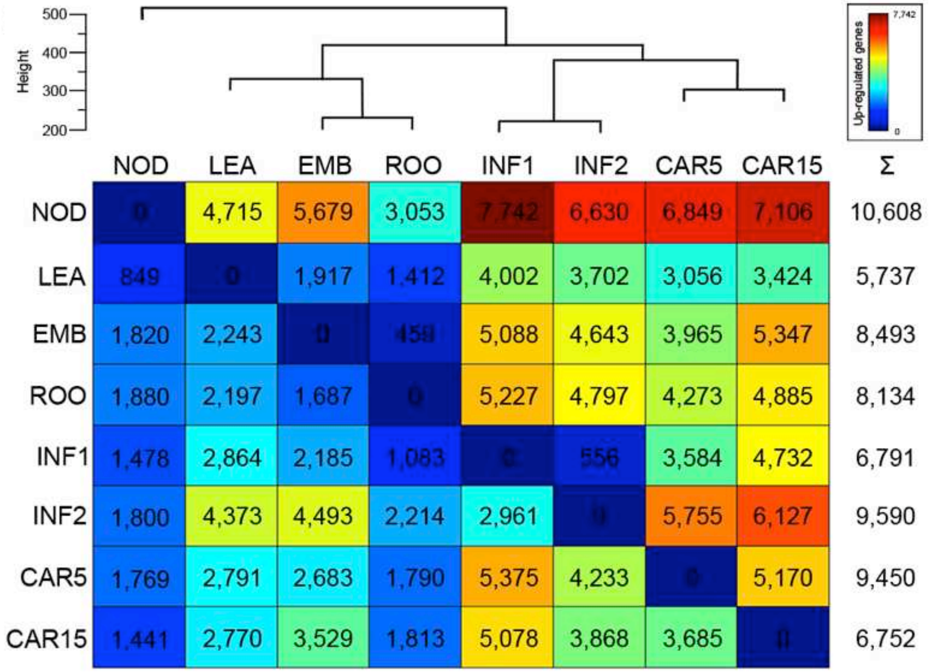
The Transcriptome of a Cereal



The Transcriptome of a Cereal

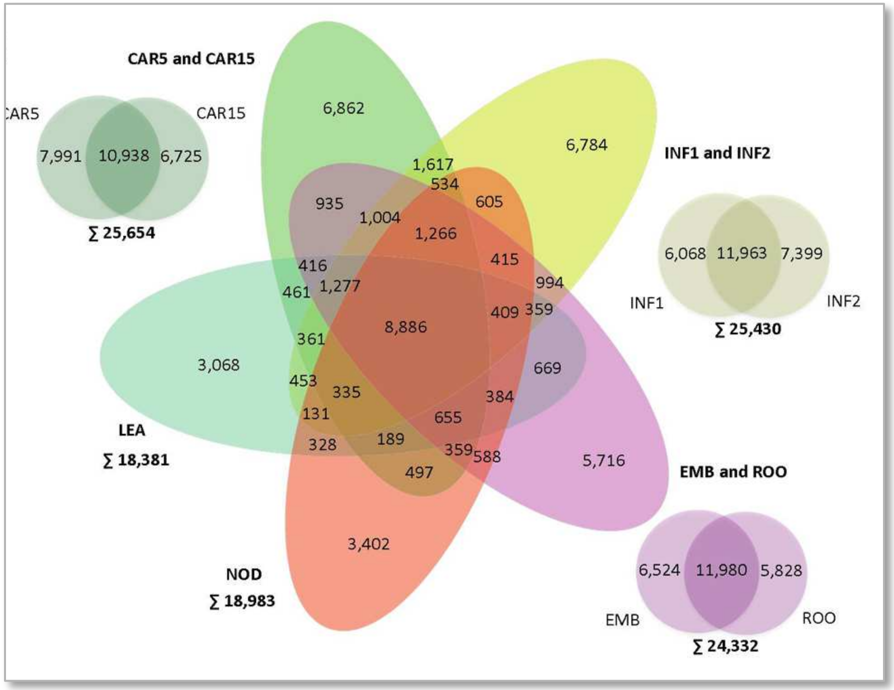
- Overlap and Dynamics in Expression -



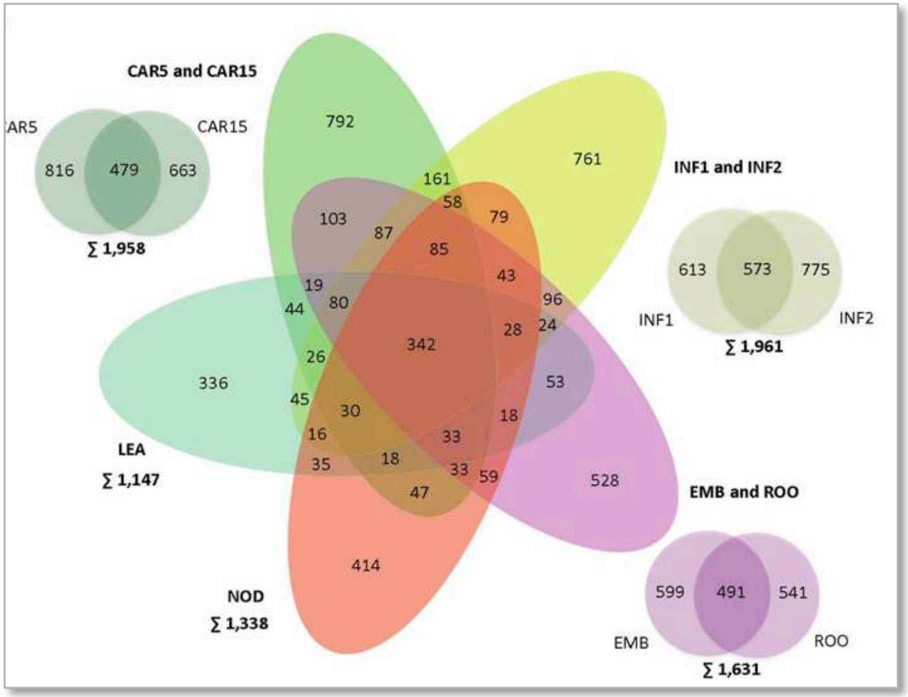


	NOD	LEA	EMB	ROO	INF1	INF2	CAR5	CAR15	Σ
NOD	0	4,715	5,679	3,053	7,742	6,630	6,849	7,106	10,608
LEA	849	0	1,917	1,412	4,002	3,702	3,056	3,424	5,737
EMB	1,820	2,243	0	458	5,088	4,643	3,965	5,347	8,493
ROO	1,880	2,197	1,687	0	5,227	4,797	4,273	4,885	8,134
INF1	1,478	2,864	2,185	1,083	0	556	3,584	4,732	6,791
INF2	1,800	4,373	4,493	2,214	2,961	0	5,755	6,127	9,590
CAR5	1,769	2,791	2,683	1,790	5,375	4,233	0	5,170	9,450
CAR15	1,441	2,770	3,529	1,813	5,078	3,868	3,685	0	6,752

The Transcriptome of a Cereal
- A pronounced RNA based Regulatory Layer (?)-



Pronounced Differential Alternative Splicing



Nonsense Mediated Decay (NMD) and Premature Termination Codons (PTC) are subject to translational and transcriptional regulation

Wheat – a WGS approach
Aims and Goals

Challenges

- hexaploid structure AABBDD
- 7 chromosomes per genome (42 chrom.)
- 17.1Gb
- ~80% repeat content

Aims

- ▶ Overview of the wheat gene content
- ▶ Gene retention rate
- ▶ Contracted / expanded gene families
- ▶ Pseudogenization
- ▶ Relationship of homeologous genes

WGS assembly using „*in silico* exon capture“

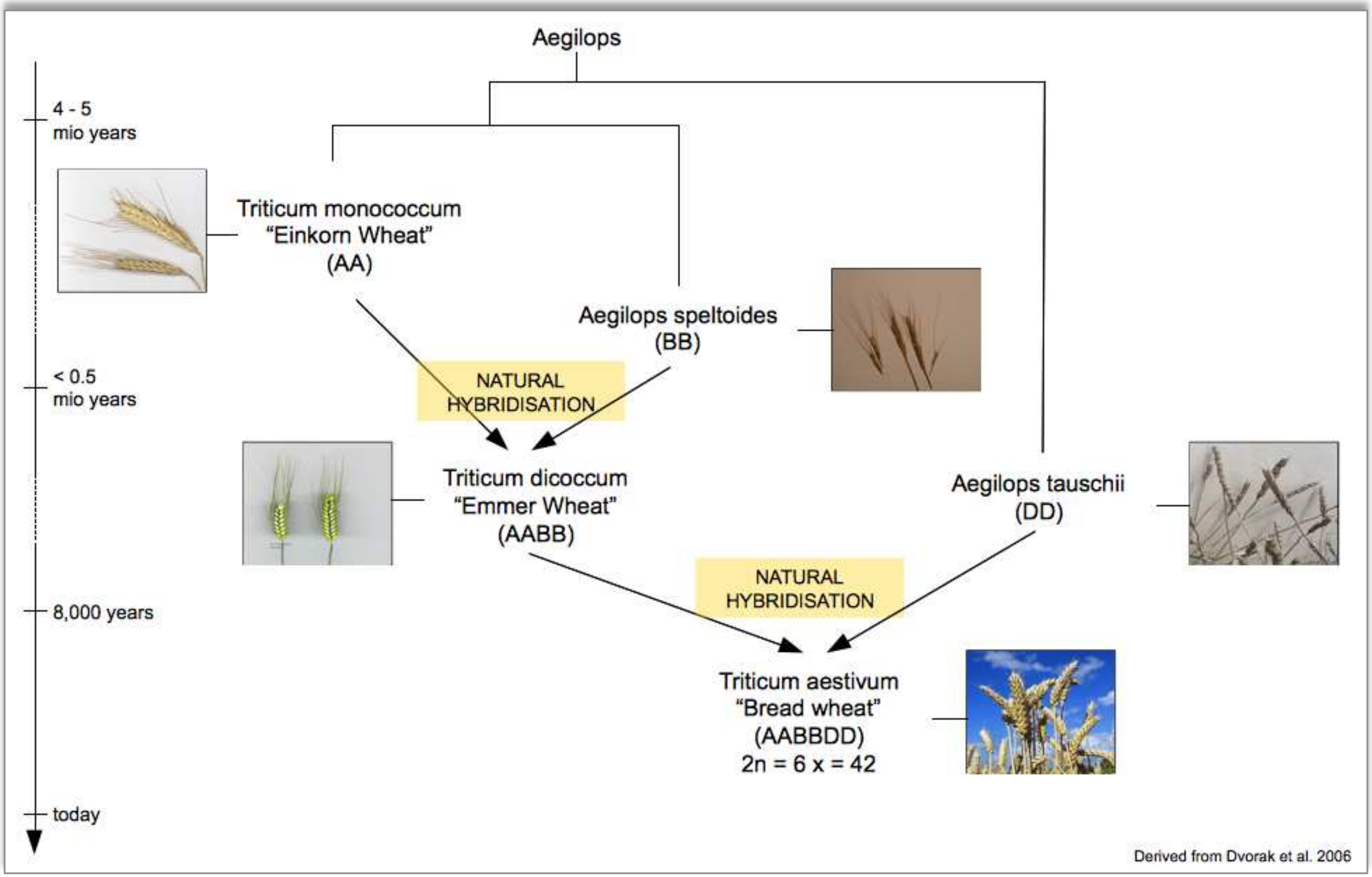
① Use fully sequences and analysed reference genomes (rice, *Brachypodium*, sorghum)

② Group genes into families (Orthologous Groups)

③ Use the orthologous group representatives as sequence baits to capture corresponding sequence reads.

④ Do sub-assembly for each „orthologous bin“ seperatly

Bread Wheat Genaology



Aegilops

4 - 5
mio years

< 0.5
mio years

8,000 years

today

Triticum monococcum
"Einkorn Wheat"
(AA)

Aegilops speltoides
(BB)

NATURAL
HYBRIDISATION

Triticum dicoccum
"Emmer Wheat"
(AABB)

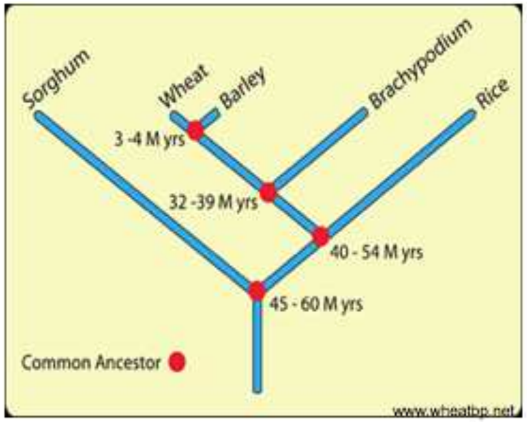
Aegilops tauschii
(DD)

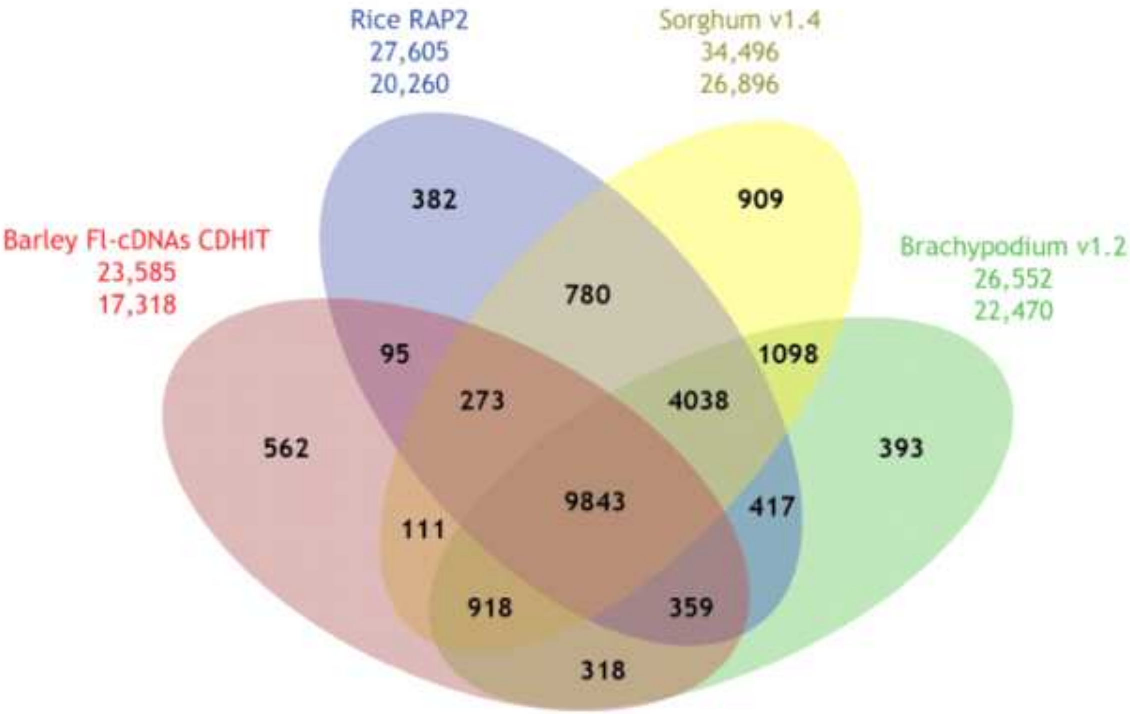
NATURAL
HYBRIDISATION

Triticum aestivum
"Bread wheat"
(AABBDD)
2n = 6 x = 42

Derived from Dvorak et al. 2006

Ortholome directed assembly circumvents limitations faced by WGS assembly



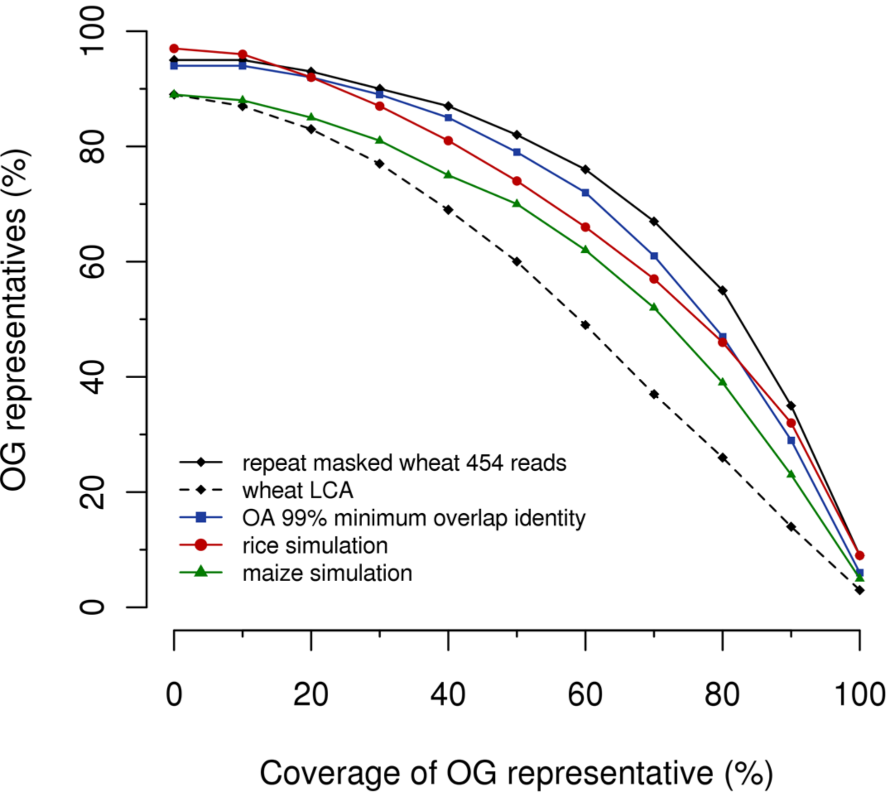


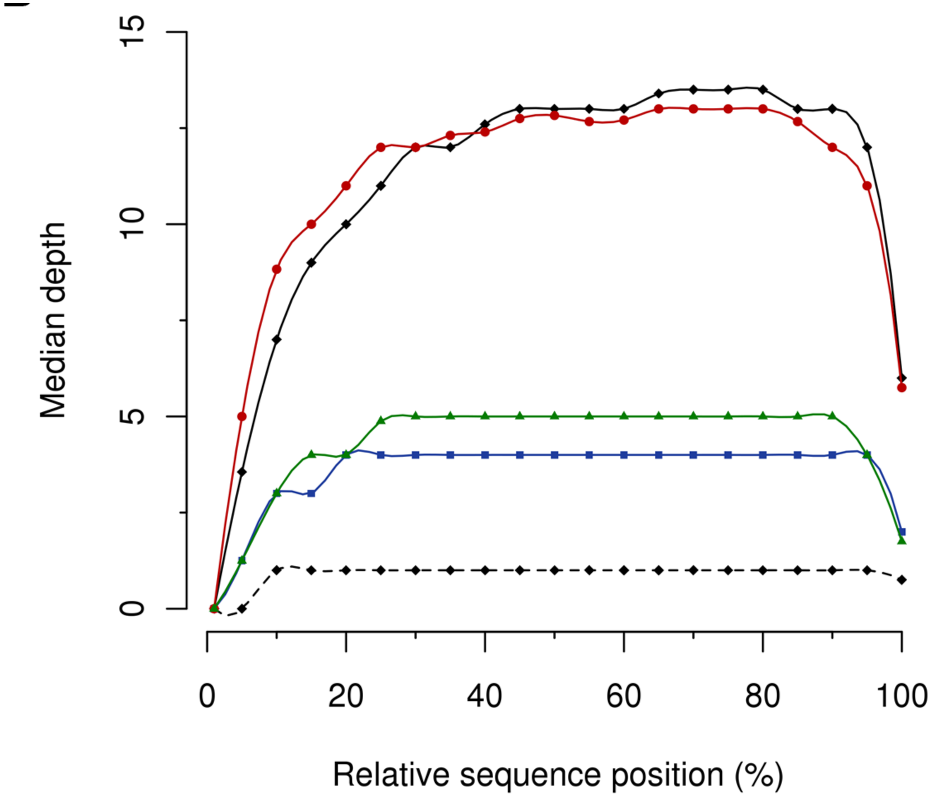
7,996 Brachypodium genes
3,582 Sorghum genes
5,337 Rice genes
5,337 Barley FL-cDNAs

20,051 orthologous gene (Og) representatives

used as templates to guide stringent assemblies

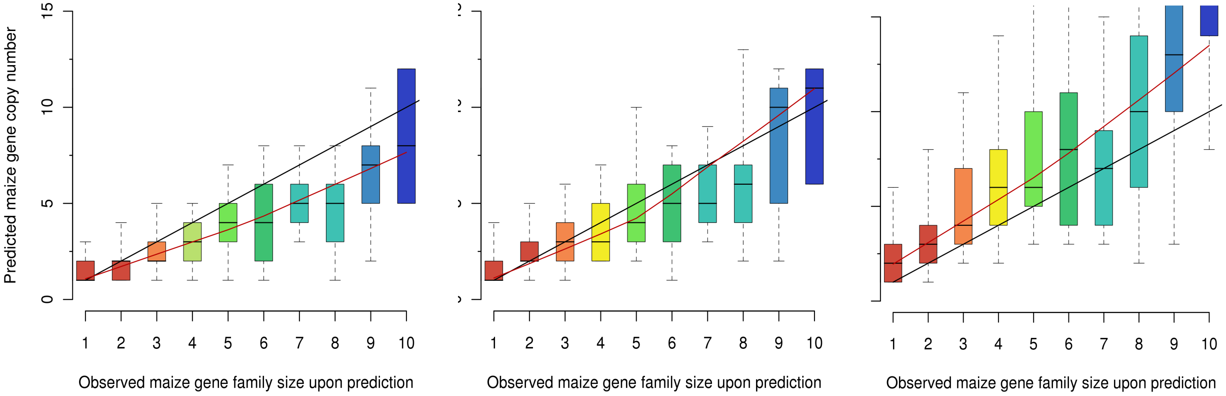
Coverage of Orthologous Group





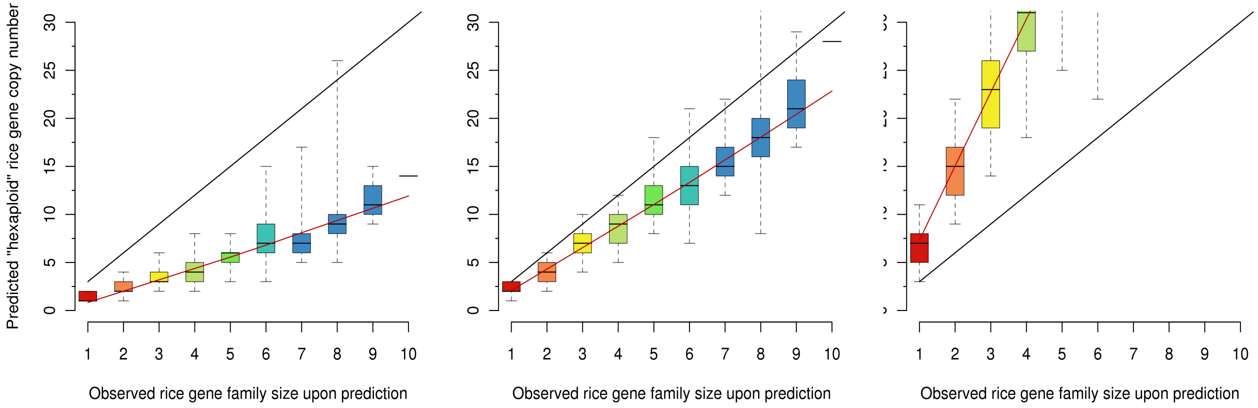
Gene Copy Retention after Polyploidization
- Calibration of the method-

Maize

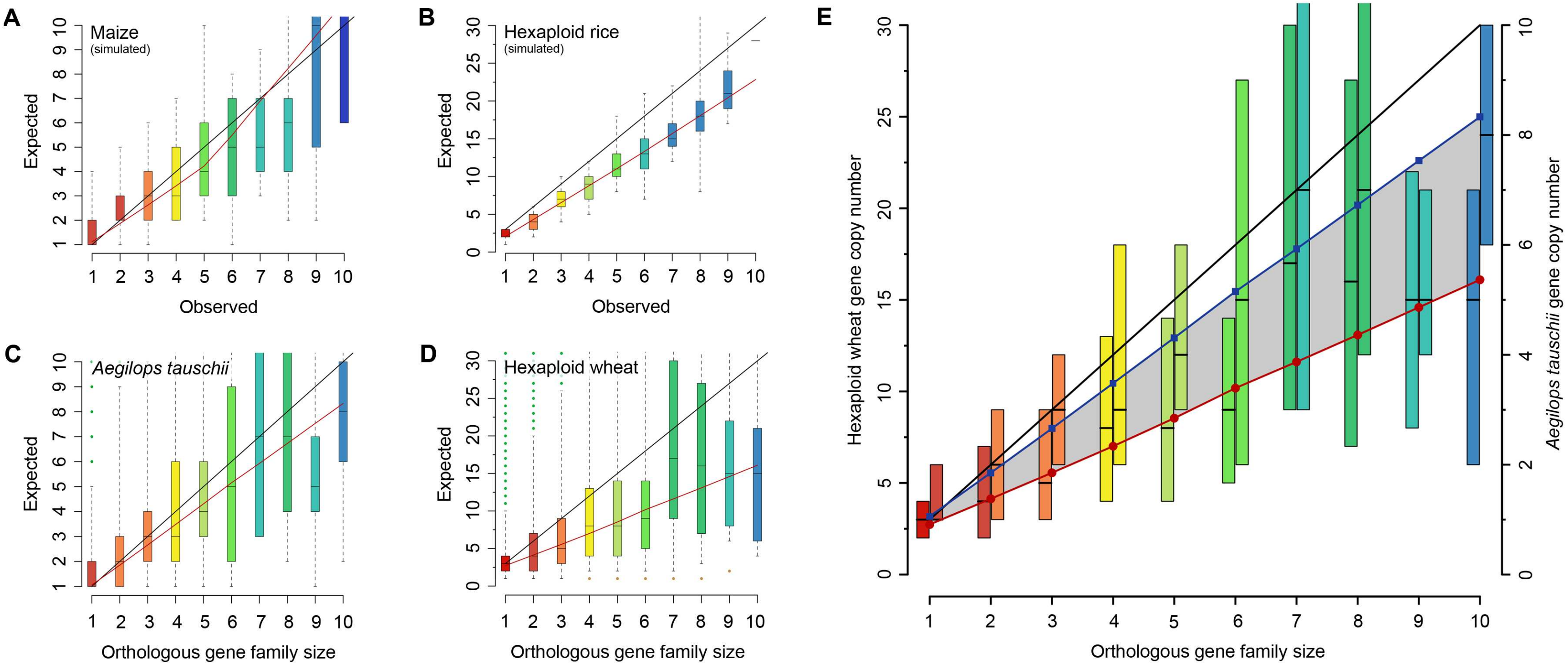


97% **99%** **100%**

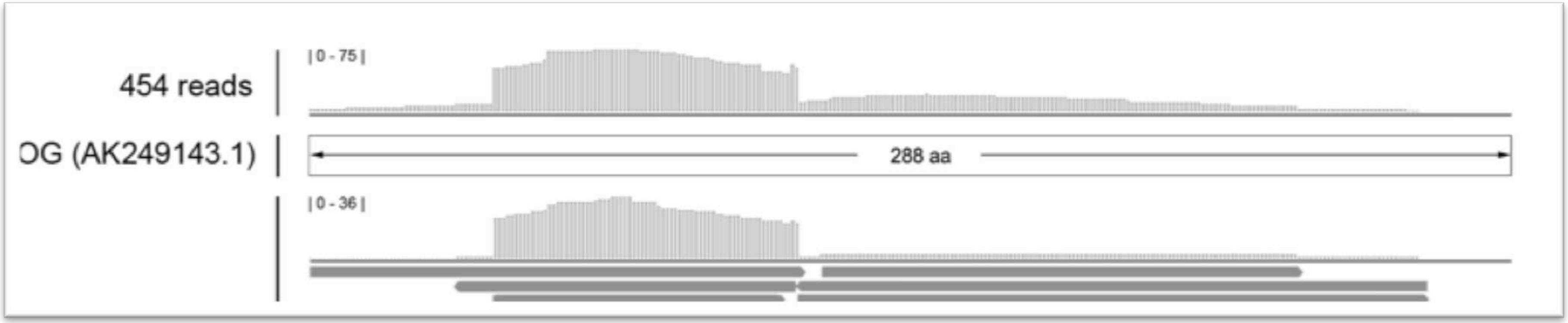
Hexaploid Rice
„TRice“



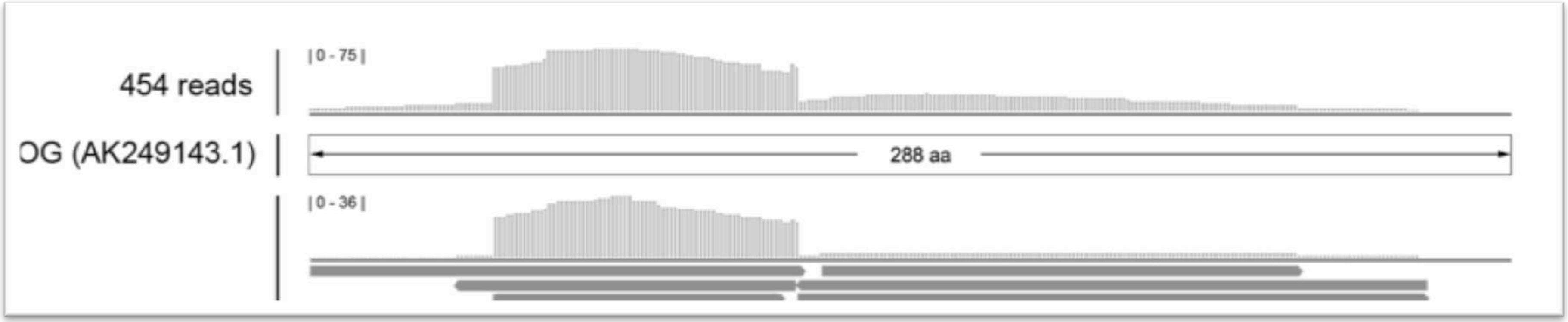
Gene Copy Retention after Polyploidization

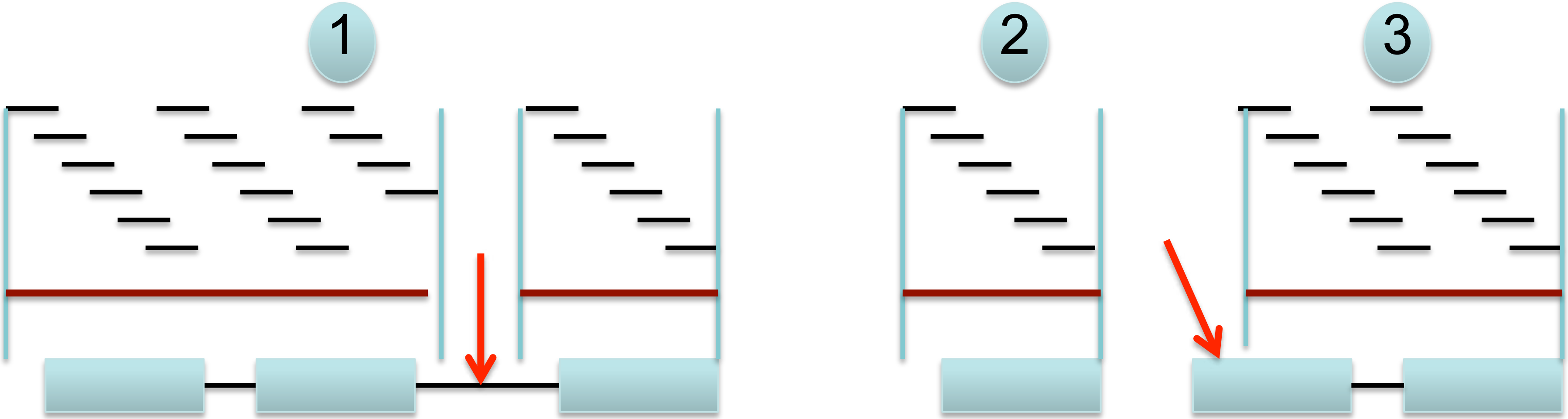


The ortholome direct assembly delivers ordered segments

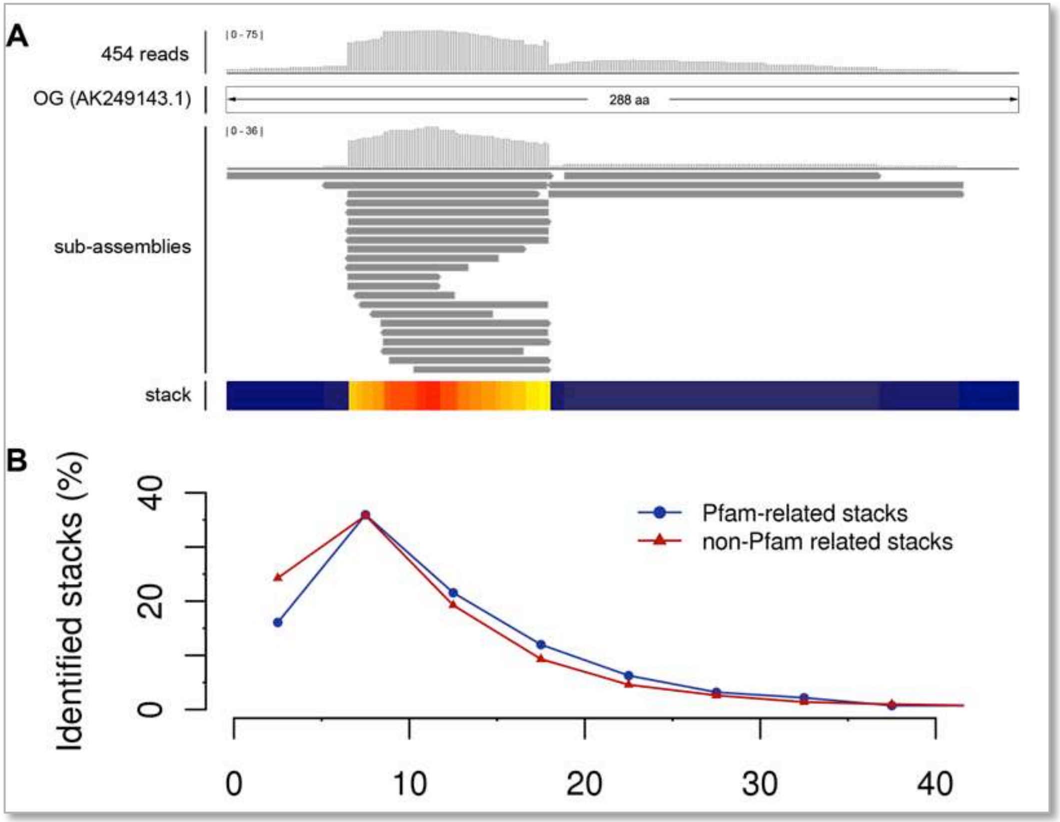


The ortholome direct assembly delivers ordered segments II

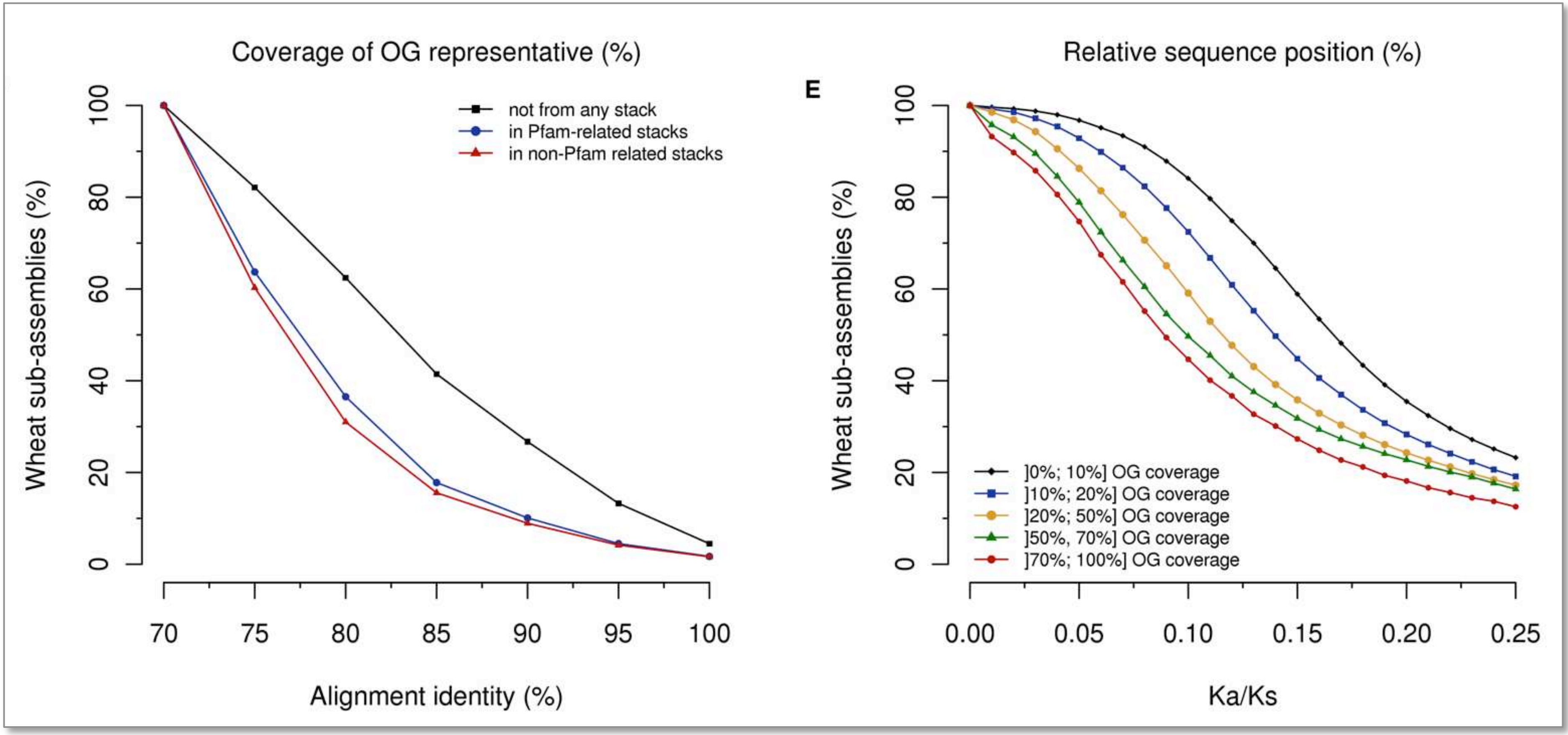




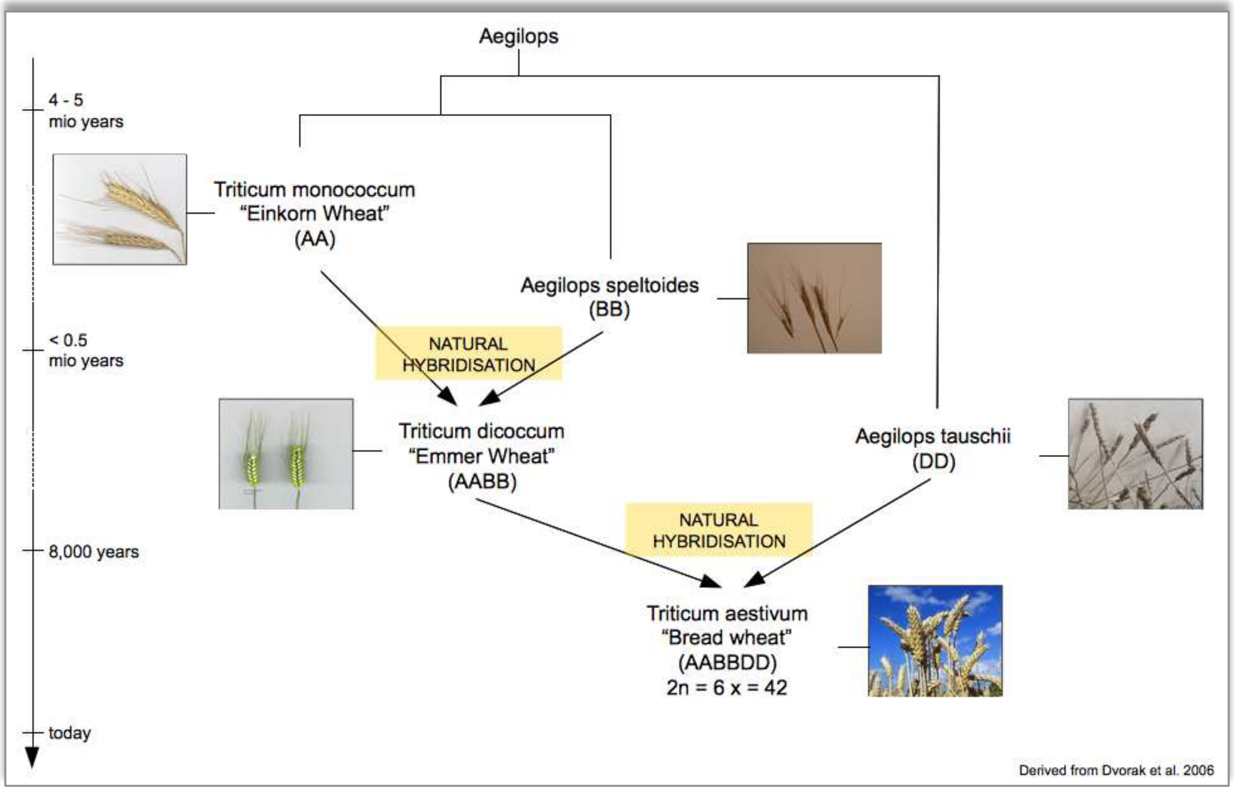
Gene fragments are abundant in wheat



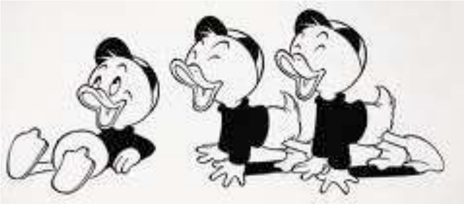
Gene fragments are abundant in the wheat genome



The Three Nephews: the A, B and D‘s of wheat



Derived from Dvorak et al. 2006

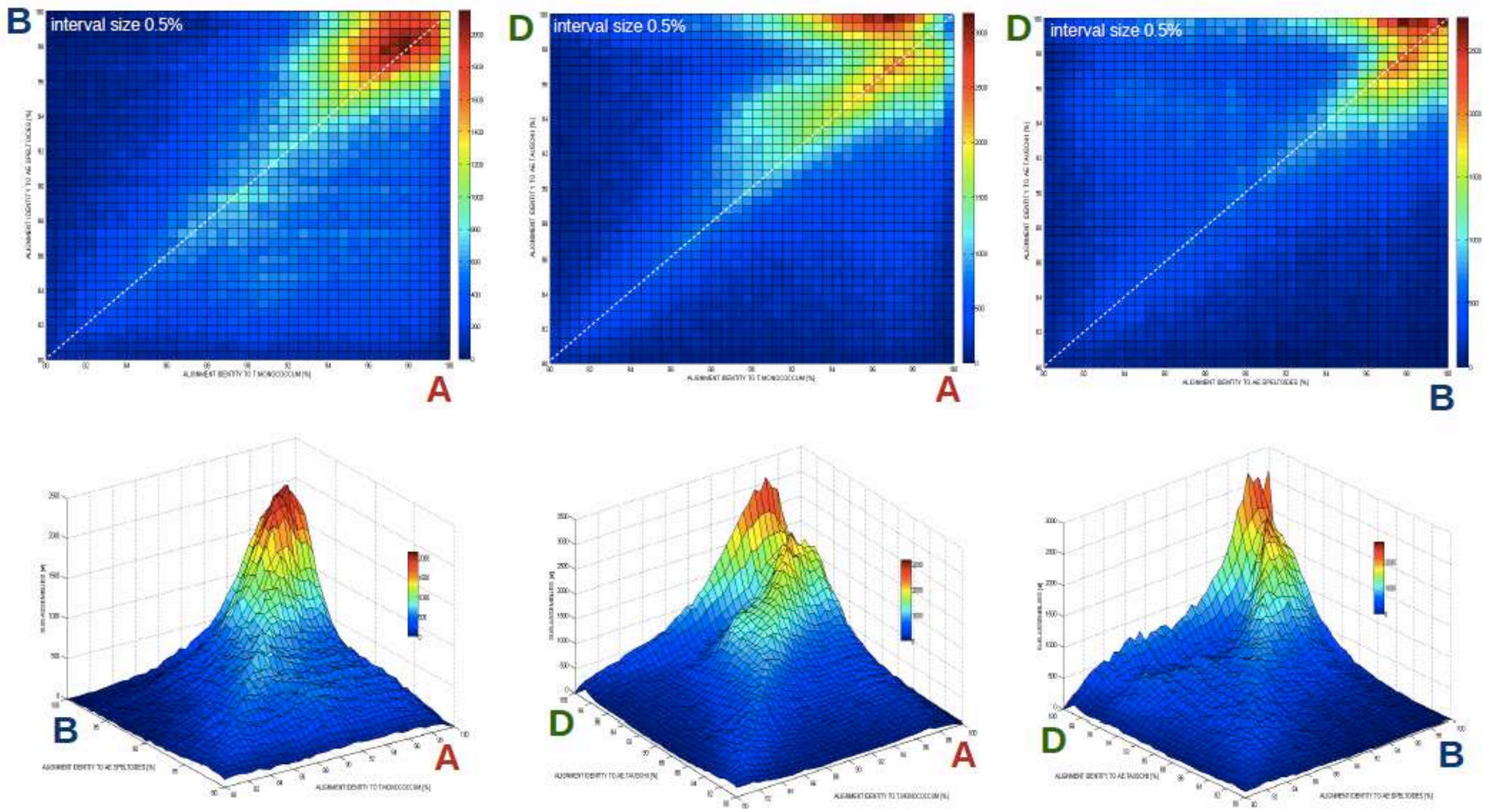


Shotguns (Illumina 80x (*T.monococcum*)) and 454 (3x (*Ae.tauschii*))

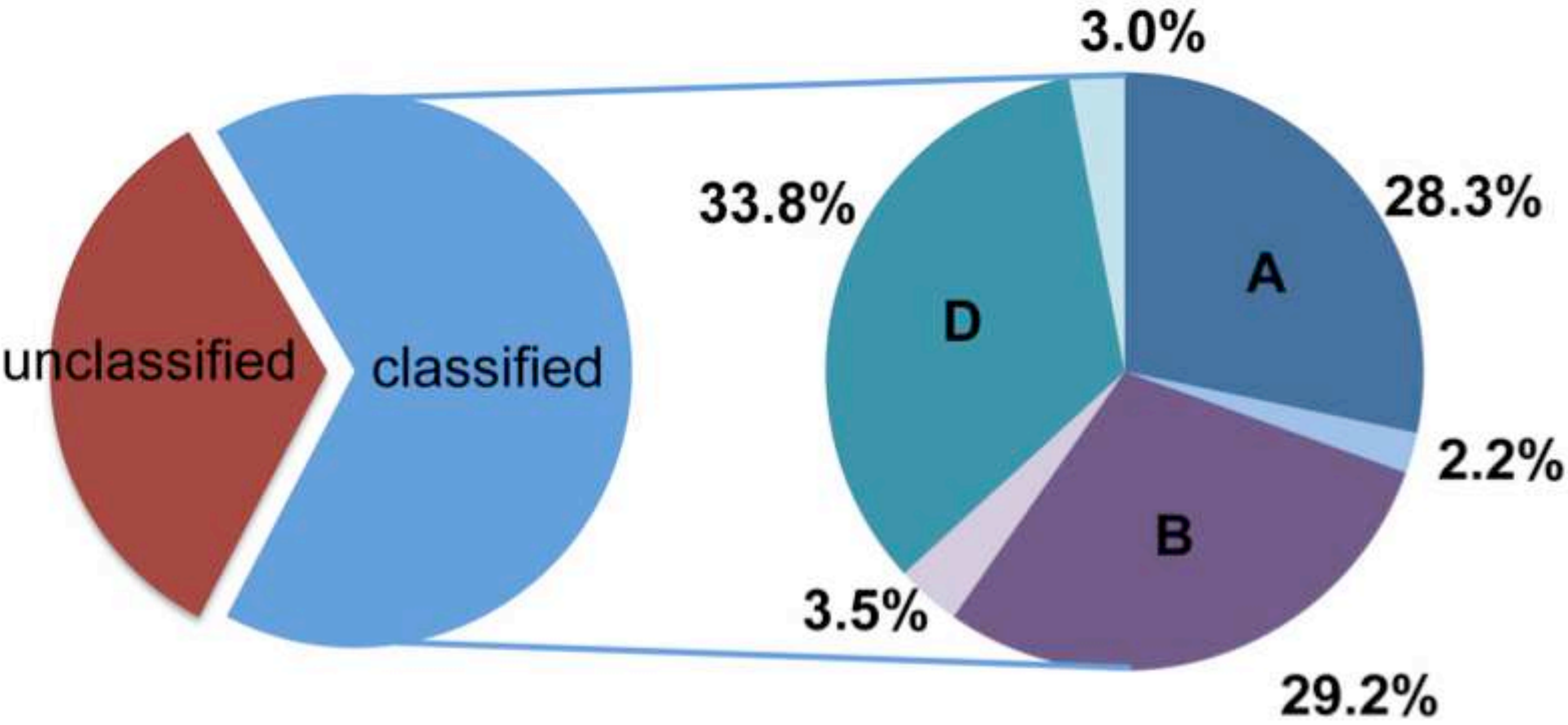
cDNA seq‘s from the *Ae. speltoides* group (B)

Can A and D genome shotgun data be used to dissect the ABD of wheat?

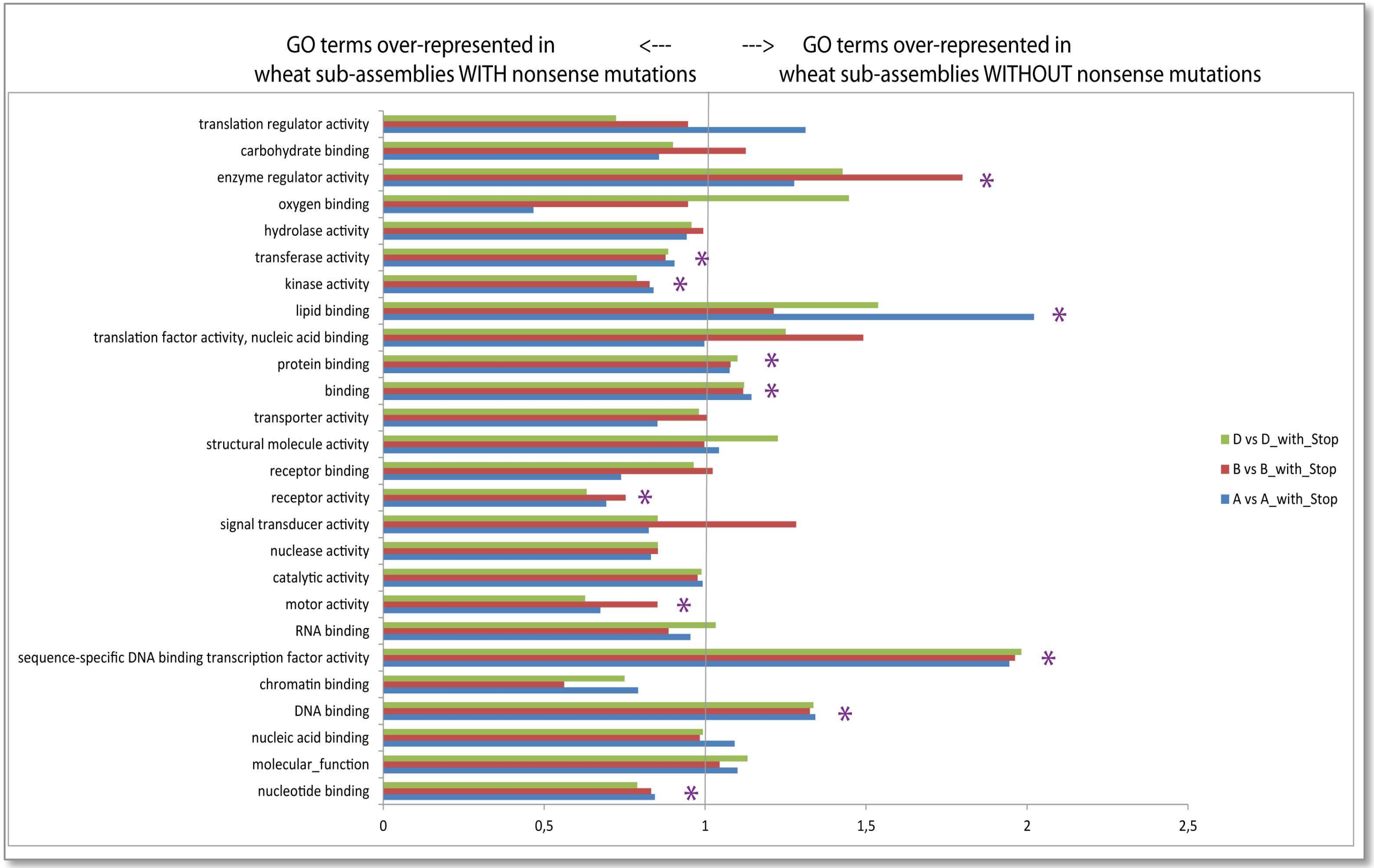
The Three Nephews: Similarity on a Sequence Basis



Wheat A, B and D Assignment using Machine Learning (SVM)



Particular Gene Categories are preferentially retained



trans PLANT

HelmholtzZentrum münchen
German Research Center for Environmental Health

Haystack -omics

Almost full gene complement detected and structured

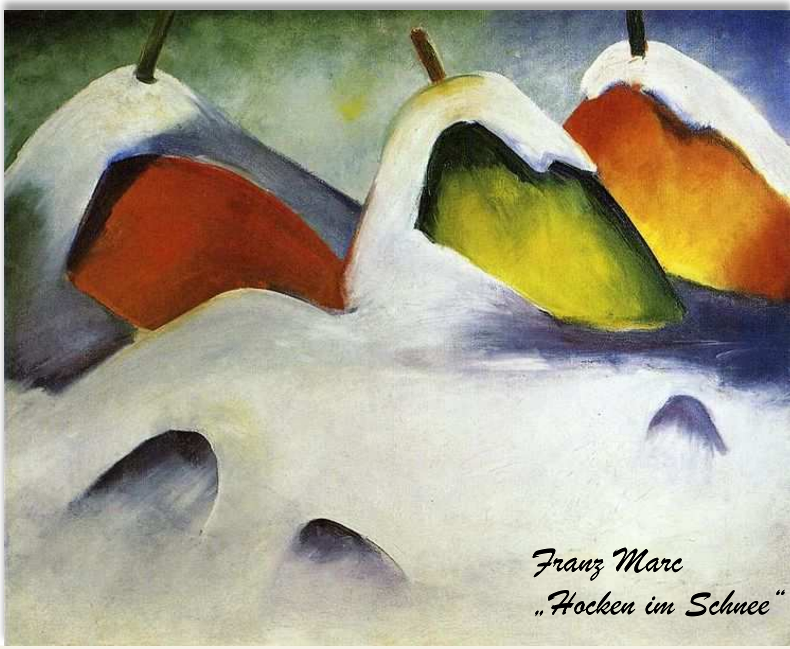
10000s of pseudogenes detected

Separation of A, B and D using machine learning with > 75% accuracy

Complementary to chromosome sorting approaches

Applicable to polyploids in general to get genome overview

Rapid and economic approach to pragmatically cope with limitations in sequence technology



Franz Marc
„Hocken im Schnee“

Take home messages

- A shortcut to access average sized plant genomes can be accessed using WGS, WCS and Genome Zipping
- Join of heterogeneous but complementary datasets delivers *Triticeae* „gene-omes“
- Provide jumboards for gene isolation, meta-analysis, genome wide functional analysis, comparative analysis, the $x10^3$ barley‘s/wheat‘s/rye‘s, ...
- B‘s are likely products of WGD/introgression events

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Bundesministerium
für Bildung
und Forschung

gabi

Deutsche
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DFG

SEVENTH FRAMEWORK
PROGRAMME

Triticeae Genome