



Wheat DArT markers: mapped and sequenced

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Introduction

We recently increased the number of markers on our wheat genotyping array to more than 5,000 (*WHEAT 2.6* array). The new markers were obtained by screening a variety of germplasm pools, including wild relatives with different ploidy levels. In collaboration with Jaroslav Dole el and his team we also added DArT markers derived from flow-sorted chromosomes (3B, 1B5) to this array in order to test the feasibility of targeting marker development to specific genome regions. The combined *WHEAT 2.6+1B5/3B* array is now being used to genotype several mapping populations. Here we report on our ongoing effort towards building a wheat consensus map. Markers on the *WHEAT 2.6* array also are currently being sequenced. We present an analysis of an initial batch of 1,144 sequences.

Mapping

In collaboration with colleagues from the wheat community, we are progressing towards building an integrated DArT/SSR consensus map for wheat. An initial survey of genetic maps built for 14 bi-parental populations (selected among almost 200 genotyped by Triticarte) led to the assignment of 2,874 markers to chromosomes (file available upon request). Approximately 7% of these markers mapped to more than a single locus (Table 1). Randomly selected single-locus markers were distributed across sub-genomes at an approximate ratio of 3:2:1 (B:A:D) (Figure 1, left panel). More markers mapped to homeologous chromosome groups 1,2,3 and 7 than to groups 4,5 and 6 (Figure 1, right panel). Markers prepared from chromosome-enriched libraries tended to map to the respective chromosomes (1B5, 3B), as shown in yellow in Figure 1 and detailed in Table 2. Markers prepared from tetraploid and diploid wheat relatives tended to map to the corresponding sub-genomes of bread wheat. Yet the efficiency of this approach of targeting markers from sub-genomes was lower for the D genome than for the other sub-genomes (Table 2). Currently, the number of markers assigned to individual chromosomes ranges from 20 (4D) to 504 (3B) (Table 3).

Table 3. Chromosome assignment*

Homeologous group	Genome		
	A	B	D
1	131	277	71
2	90	194	77
3	95	504	89
4	165	61	20
5	85	158	27
6	877	131	34
7	149	181	58

* for single-dose markers

Table 4. Saturating chromosome 3B

	Increase in No. of markers or loci	
	Pop.1	Pop.2
markers	+171% (113→306)	+176% (78→215)
genetic loci	+89% (45→85)	+90% (40→76)

The objective of saturating chromosome 3B with DArT markers was to improve the efficiency of anchoring 3B BAC contigs from Chinese spring to DArT genetic maps. Table 4 shows that by using flow-sorted chromosomal DNA as a starting material, we could increase the number of 3B markers by 170%, while almost doubling the number of unique genetic loci.

More populations are now being genotyped on the *WHEAT 2.6* array. Those that are highly polymorphic and, ideally, have also been genotyped with SSR markers, will be selected for building a consensus map. Currently, this set contains four populations (Figure 2); but more will be added as data become available. Figure 3 displays an alignment of the DArT maps for two highly polymorphic populations as an example. The provenience of markers (see Table 2 for examples) is encoded by colours. Clearly, the 1B/1R translocation segregates in population 2, as visible from the large blocks of co-segregating wheat and rye/triticale-derived markers. Chromosome 7A displays an extreme example of unequal recombination frequencies in different crosses: a 171-cM regions spanned by a pair of markers in population 2 is compressed to only 3 cM in population 1! We have observed other, though less severe cases of recombination frequency differences between other populations. These observations suggest to us that the standard techniques for building consensus maps will not be applicable to the wheat situation.

DArT marker sequences

A first batch of 1,144 marker sequences was obtained and subjected to clustering to identify the degree of true marker redundancy (as opposed to redundancy based on segregation patterns, which may have biological reasons). The 1,144 markers were distributed into 955 clusters (16.5% redundancy). Figure 4 suggests there is opportunity for a substantial further increase in the number of markers on the DArT array.

A BLAST search against NCBI reference sequences suggested that 26% of all markers were located in actively expressed genome regions (Figure 5, $e < 10^{-6}$). This number, while somewhat lower than in barley (35%), still confirms the 'methylation filtration' effect of the *Pst*I restriction enzyme. Only 0.1% of the markers contained organellar sequences, 0.3% produced a hit against a database containing Triticeae repeats, and 1% contained an SSR.

Outlook

- Work on the consensus map in collaboration with the wheat community is ongoing.
- Marker sequencing will be completed soon, and a database with marker sequences is going to be published.
- DArT markers are being anchored to the physical map, as done already for chromosome 3B in collaboration with Cathrine Feuillet and Jaroslav Dole el.
- The marker density on the D genome is going to be increased by isolating markers derived from flow-sorted, D genome-enriched DNA of bread wheat.

Acknowledgements

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Figure 3. Alignment of two wheat maps

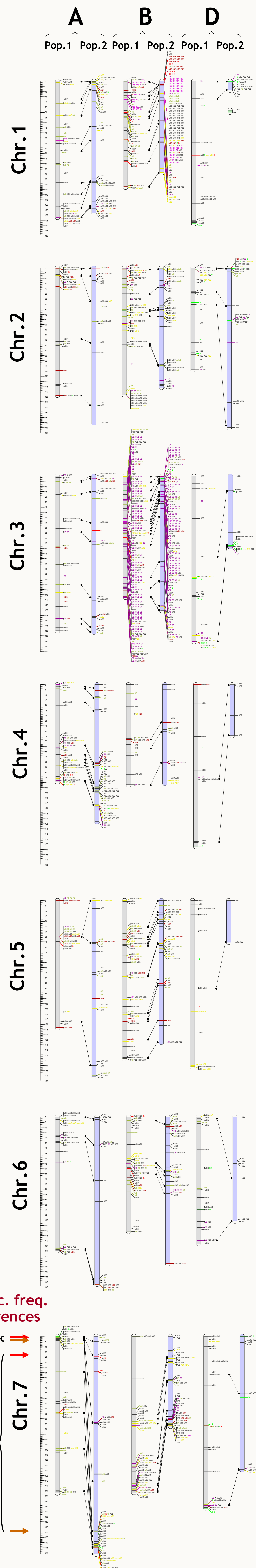


Table 1. Multi-locus markers*

No. loci/marker	No. markers	%
1	2684	93.4
2	184	6.4
3	6	0.2

* based on a survey of 14 maps

Table 2. Targeting markers to genome regions

Marker source	Source type	Mapped to targeted region	
		Pop.1	Pop.2
1B5	flow-sorted chr.	71% (17/24)	95% (40/42)
3B	flow-sorted chr.	77% (193/250)	77% (138/180)
A	A-genome species	68% (13/19)	68% (13/19)
AB	AB-genome species	98% (199/203)	97% (144/149)
D	D-genome species	79% (31/39)	56% (14/25)

Figure 1. Genome distribution of DArT markers

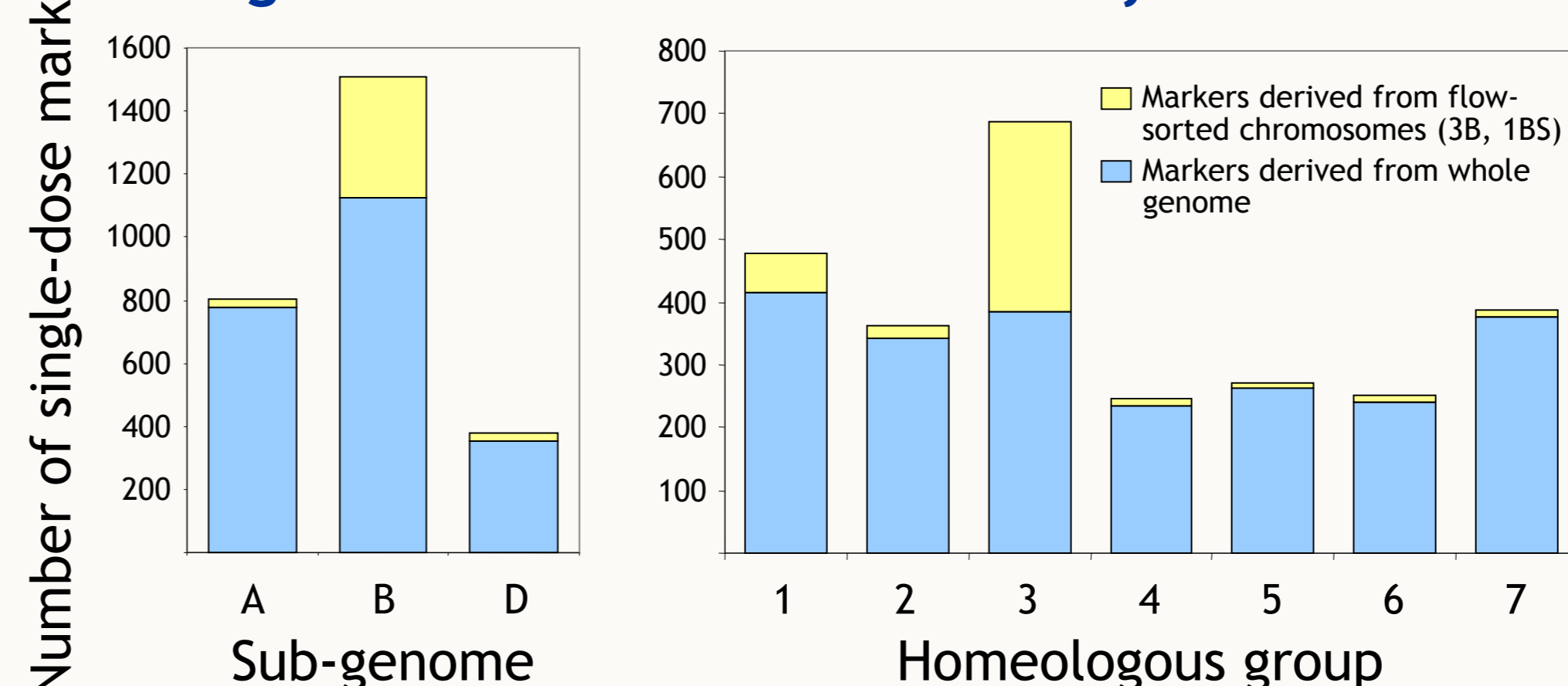


Figure 2. Markers bridging among mapping populations

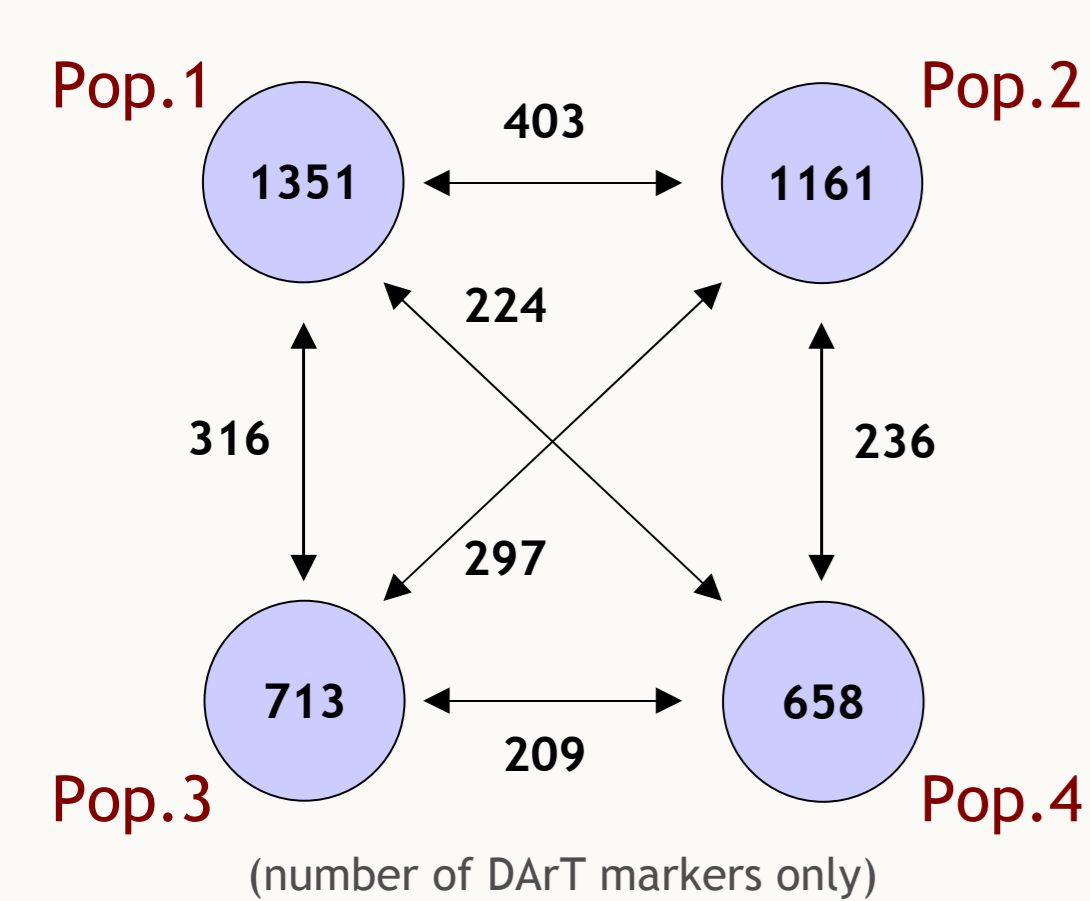


Figure 4. Clustering of marker sequences

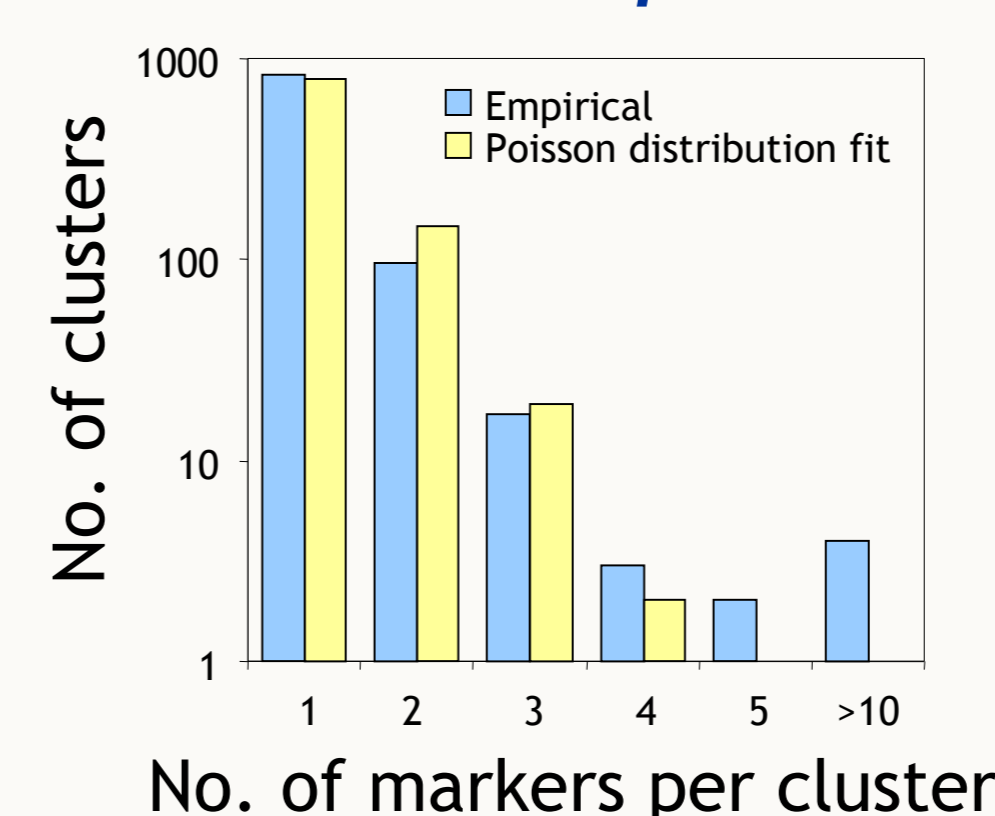
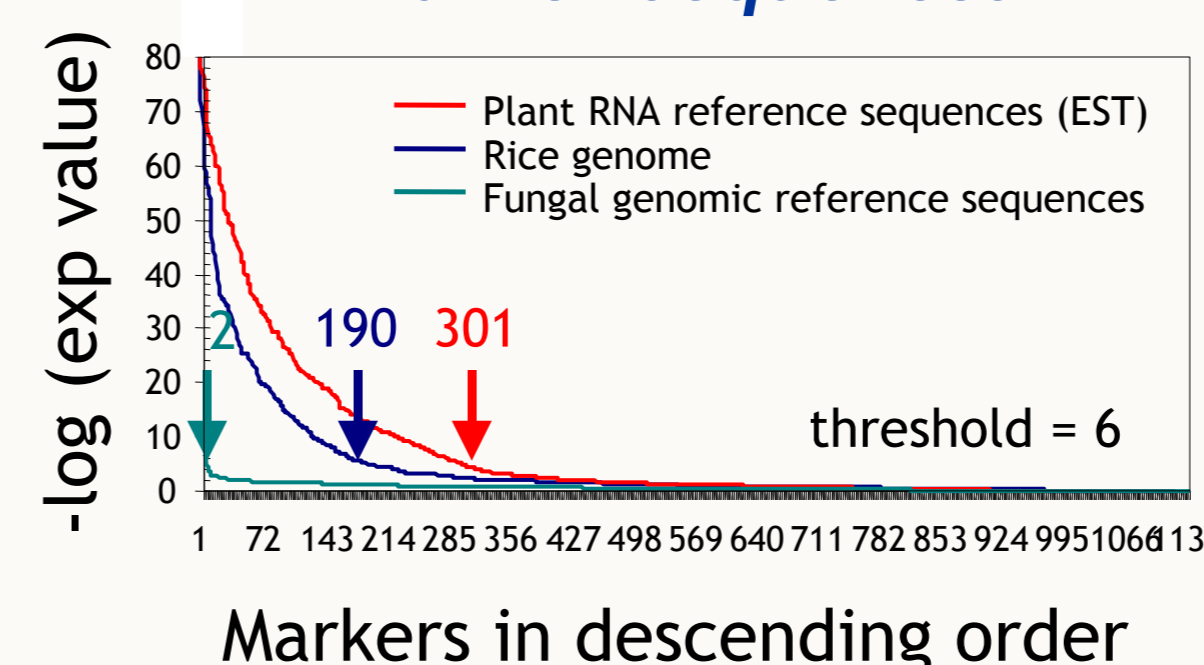


Figure 5. BLAST analysis of marker sequences



7A: rec. freq. differences

