Using sequenced mutant populations to improve wheat

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Seed Central, October 12 2017

UCDAVIS PLANT SCIENCES

hhmi Howard Hughes Medical Institute

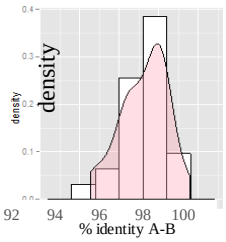
USDA NIFA United States Department of Agriculture National Institute of Food and Agriculture

Origin of the polyploid wheat species

AA ***Triticum urartu***
2x, n=7

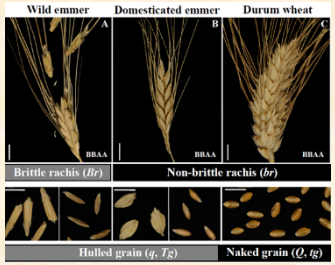
BB **Section *Sitopsis***
2x, n=7

A *vs* **B**
97.3% =



5-7 MYA

~500,000 y



AABB ***Triticum turgidum***
4x, n=14

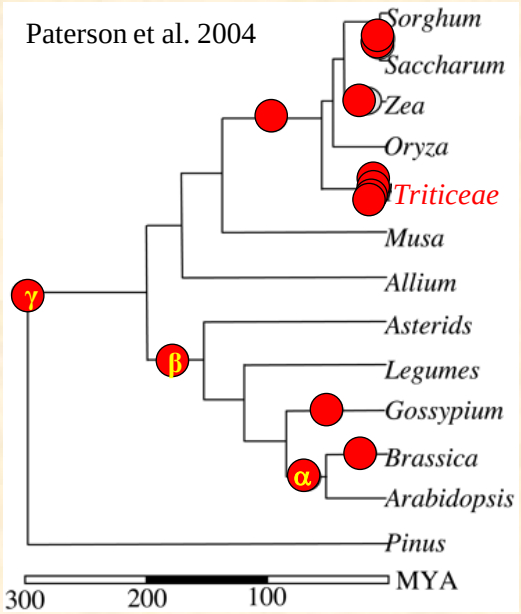
DD ***Aegilops tauschii***
2x, n=7

AABBDD ***Triticum aestivum***
6x, n=21

~10,000 years

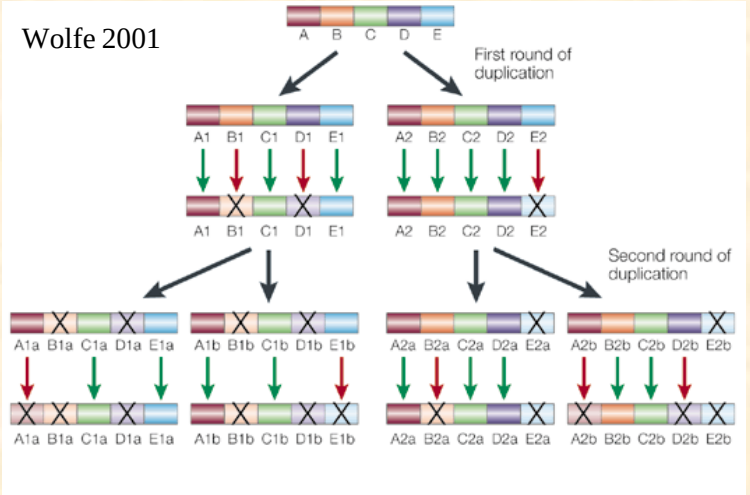
Wheat polyploid species are relatively young and there is a high level of functional redundancy among homeologs!

Plant genome landscape



Polyploidization

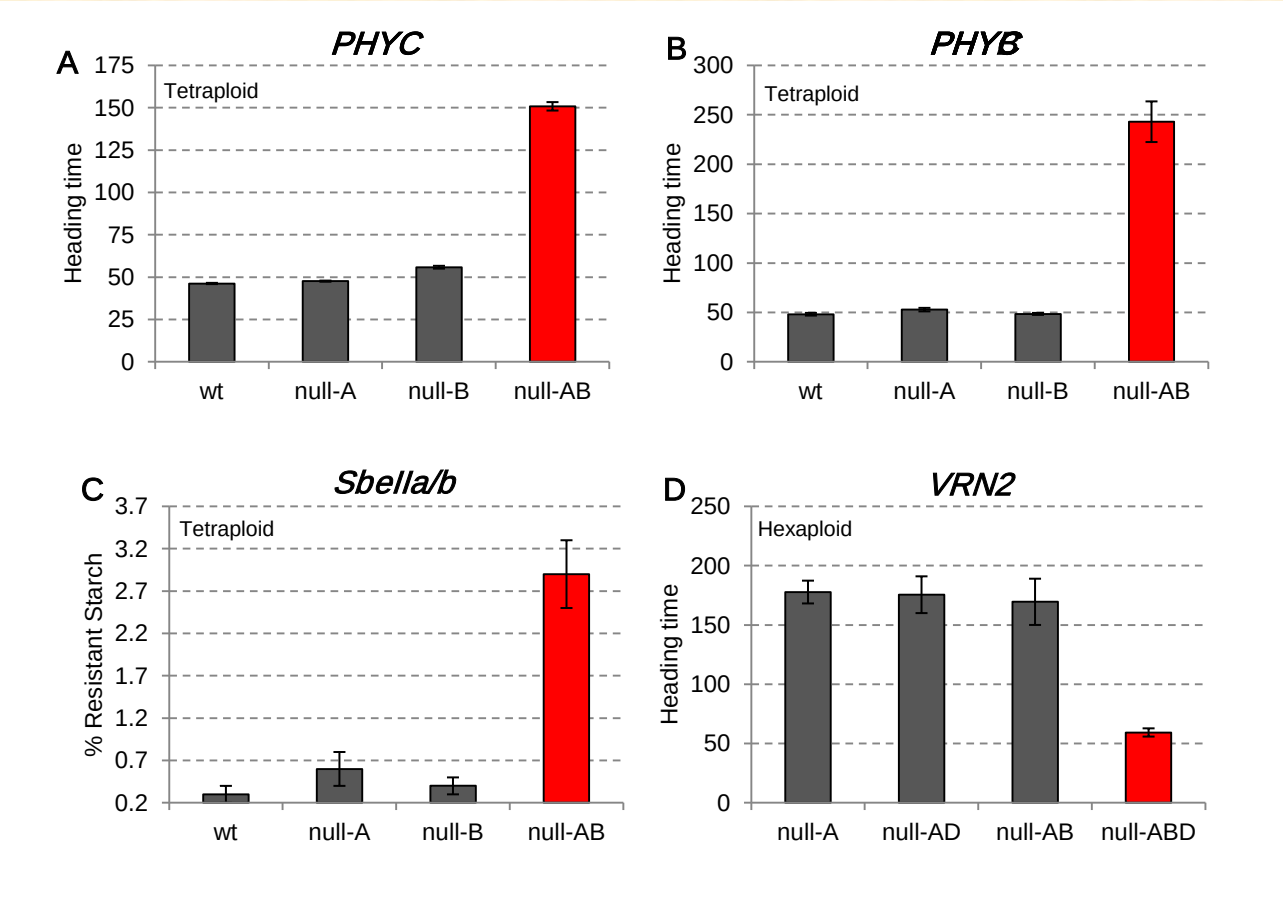
Diploidization



Diploid-like species

Erosion

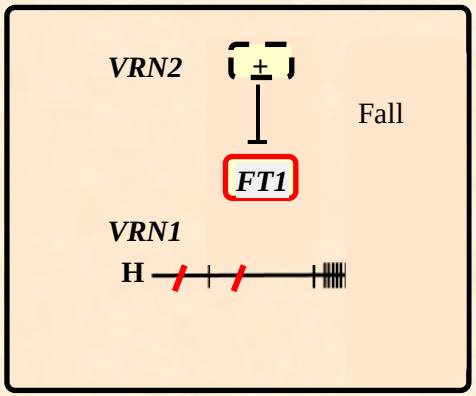
Masking effect of redundant homoeologs



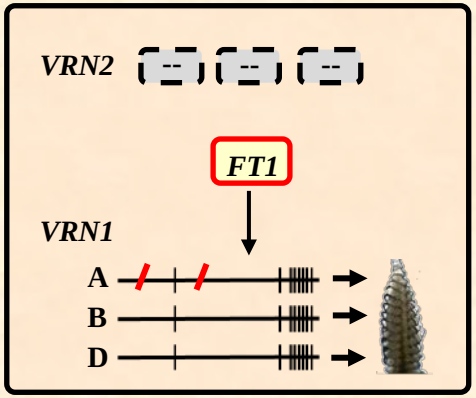
In a young polyploid the effect of many recessive mutations is usually masked by homoeolog functional redundancy from natural or human selection

SELECTION OF DIFFERENT TYPES OF VARIATION IN DIPLOID AND POLYPLOID SPECIES

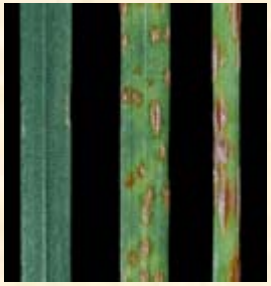
Diploid
(Barley; *T. monococcum*)

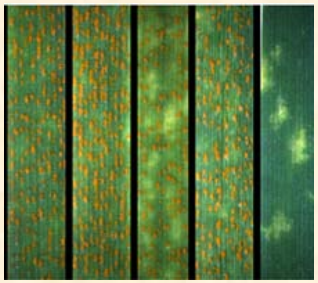


Polyploid
(bread wheat)



Rust resistance genes





Diploid barley

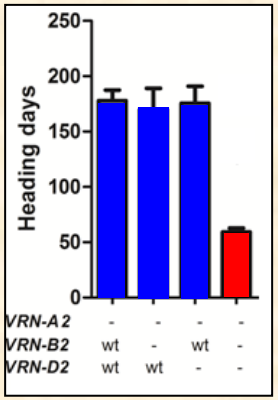
Spring accessions have
- Recessive *vrn2* alleles ~40%
- Dominant *Vrn1* alleles ~60%

Diploid wheat

Spring accessions have
- Recessive *vrn2* alleles 80%
- Dominant *Vrn1* alleles 20%

Hexaploid wheat

- No natural spring accession associated with *vrn2* reported.
- Mora than 10 *Vrn1* dominant alleles have been identified



Variation combined by MAS

Combination of three recessive *vrn2* mutations is spring

Kippes et al. 2016. TAG 129:1417

Diploid barley

- Recessive resistant alleles 26%

Hexaploid wheat

- Recessive resistant alleles 7%

***P* = 0.0007**

Ann Rev. Genet 2017 51: on line first

In young polyploid species the effect of many recessive alleles can be masked by gene redundancy

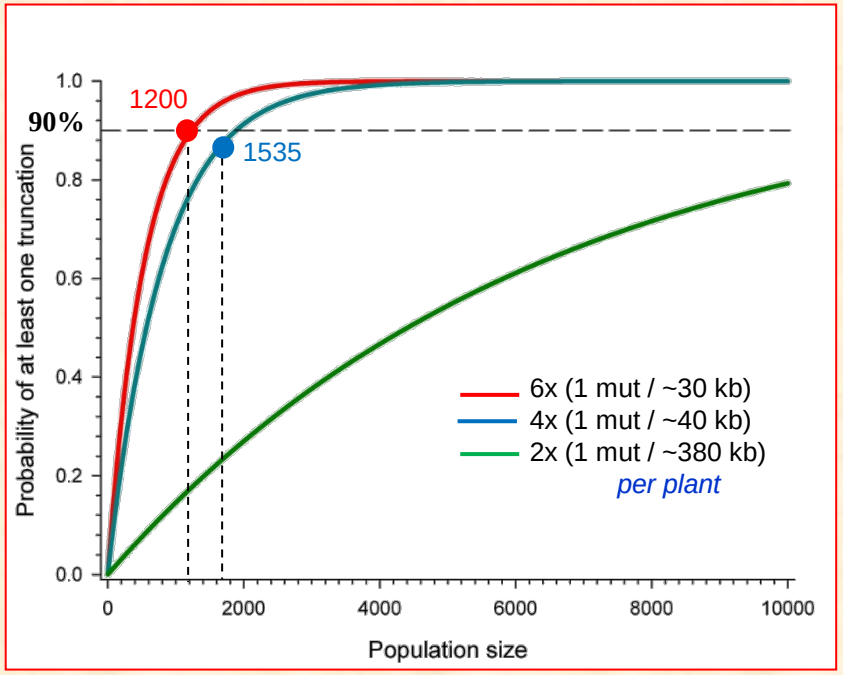
Gene redundancy and hidden variation

Potential disadvantages of gene redundancy

- Recessive mutations are masked by gene redundancy.
- Forward genetic screens do not reveal many mutations in polyploid wheat.
- Recessive variation is frequently hidden from natural or human selection.

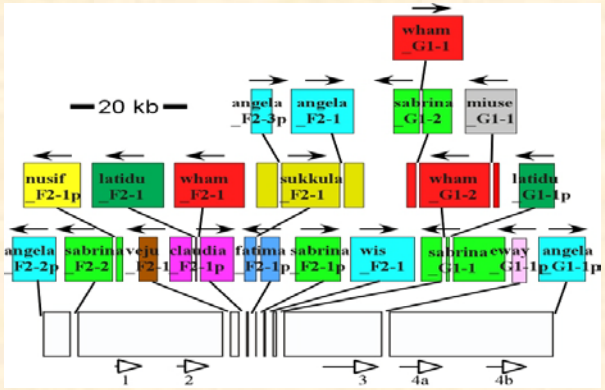
Potential advantages of gene redundancy

- Young polyploids tolerate high mutation densities.
- A high mutation density reduces the cost of sequencing complete mutant populations.
- Reverse genetic tools can expose recessive variation previously hidden to natural or human selection.



Wheat exome capture

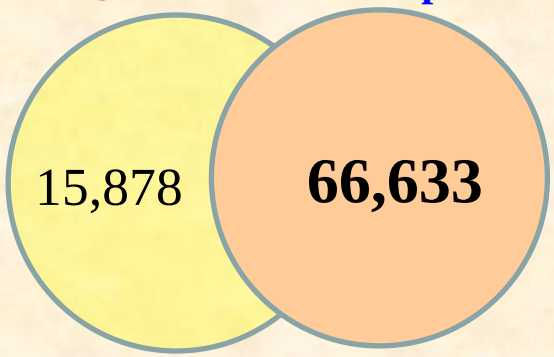
The wheat genome had a large expansion due to transposable elements



A. thaliana
- 135 Mb
- 55% repetitive

T. t. ssp. durum
- Genomes AABB
- 13,000 Mb
- **98% repetitive**

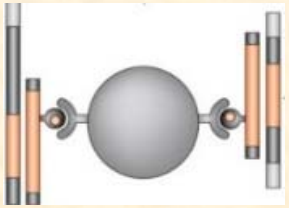
- 1.1 million wheat ESTs
- NCBI nr wheat genes
- *T. aestivum* full length cDNAs
- 4 *T. aestivum* transcriptomes
- Homologs to barley genes
- Annotated genes from our labs



Total 82,511 genes

- **Exome Capture**

Collaboration with NimbleGen

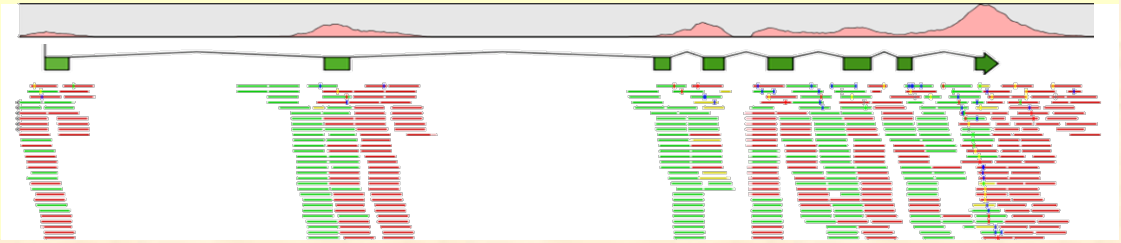


Magnetic beads carrying streptavidin, capture biotinylated probes

Public wheat exon capture
PNAS 2017 114:E913

Wheat exome capture

- Aligned transcripts to current draft IWGS A+B genome sequence
- **286,800 exons** (76% padded with 30 bp of genomic sequence)
- We optimized the α design based on 43 captures and generated a **β design** that is publicly available
- **Exon capture is very efficient**
- 84 Mb probes:
  - **119 Mb** captured in tetraploid
  - **162 Mb** captured in hexaploid.



Optimizing mapping references

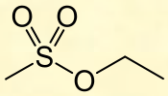
	Design seq.	IWGSC Genomic A+B	Genomic A+B+KUR
% of reads mapped	**58%**	**91.8%**	**98.2%**

- **Kronos Unmapped Reference:** we took unmapped reads from 48 Kronos lines and assembled them into 40,975 contigs (33.8 Mb).
- The addition of the KUR contigs to the A+B genome reference increased the % or mapped reads (6.4%) and reads mapped in pairs (5.7%)..

PNAS 2017 114:E913

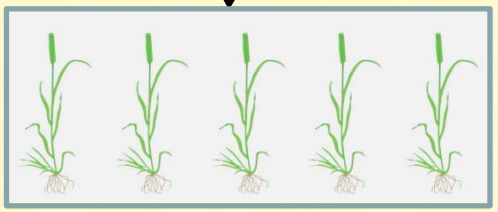
Exon capture re-sequencing of wheat EMS mutant populations

EMS treatment



M1

M2

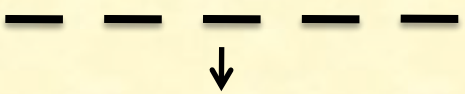


Genomic DNA

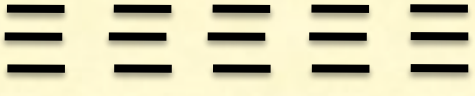
~ 1,535 lines (4x)
~ 1,200 lines (6x)

C. Uauy, 6x
JIC-UK

Genomic DNA



Sheared DNA



Barcoded library

Exome capture

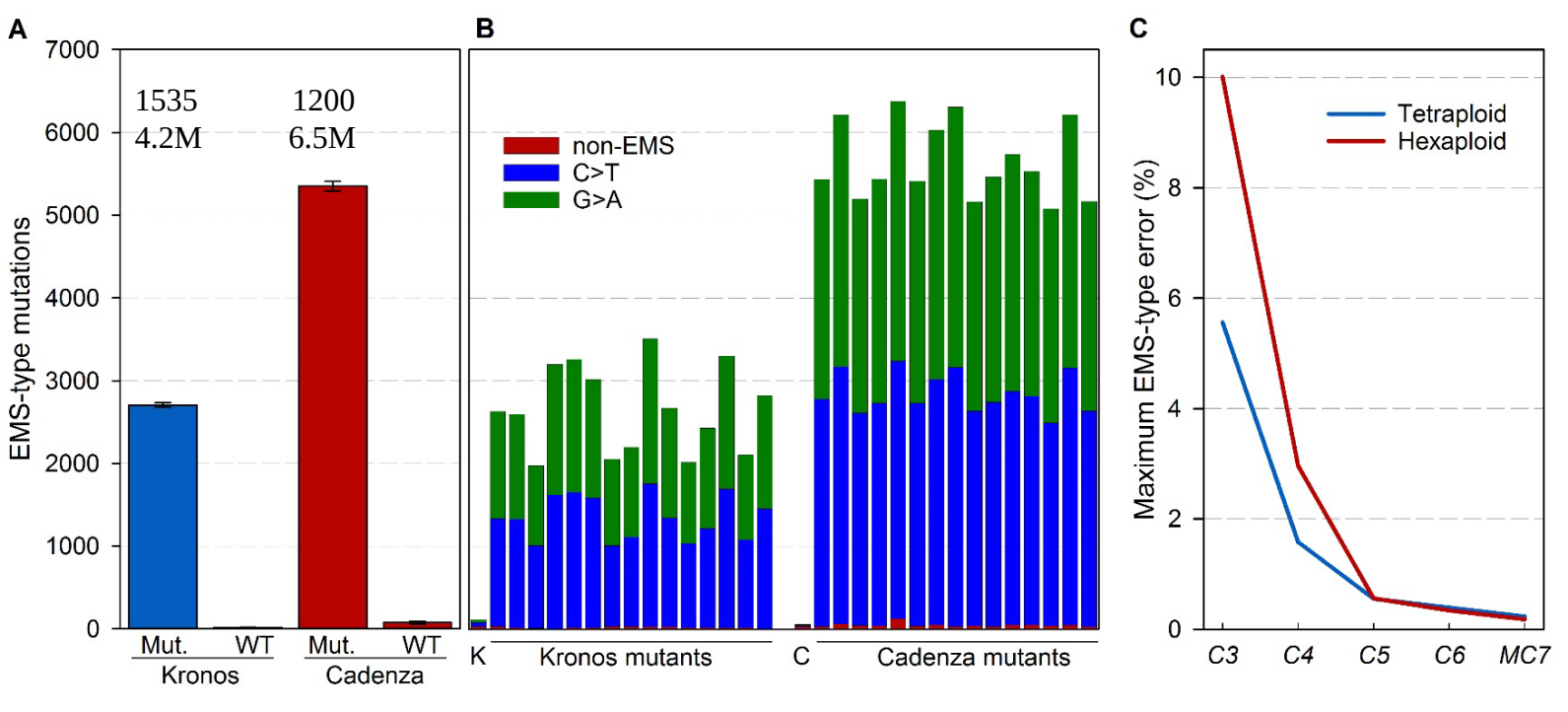
Illumina sequencing

ATGT	ATGC	ATAC	ATGC	ATGC
TACT	TGTT	TGCT	TGCT	TGCT
CGTG	AGTG	CGTG	TATG	CGTA

Mutation identification

ATG**T**	ATGC	AT**A**C	ATGC	ATGC
T**A**CT	TG**T**T	TGCT	TGCT	TGCT
CGTG	**A**GTG	CGTG	**T**GTG	CGT**A**

Characterization of mutations in tetraploid wheat



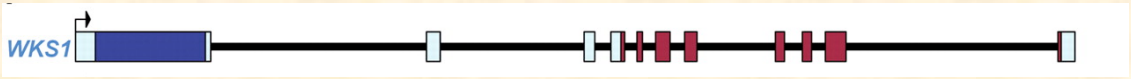
- Sequencing coverage 27-30X. Adapted MAPS mutation calling pipeline developed by Luca Comai.
- **>10,000,000** mutations in wheat coding regions (99.8% accuracy).
- **>90%** of the wheat genes have truncations or loss-of-function mutations.

User possibilities (left panel of web site)

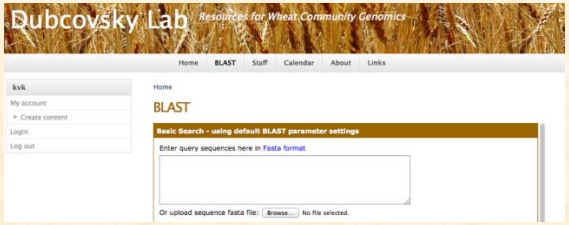
- Default is 99.8% accuracy (HetMC5/ HomMC3)
- User can see another 5,000,000 mutations at >90% accuracy (HetMC3 /HomMC2)
- A red line below the mutants indicate mutations mapped to multiple sites

Wheat TILLING database: BLAST tool at UCD -JBrowse

1. Identify gene of interest



2. Blast our public database

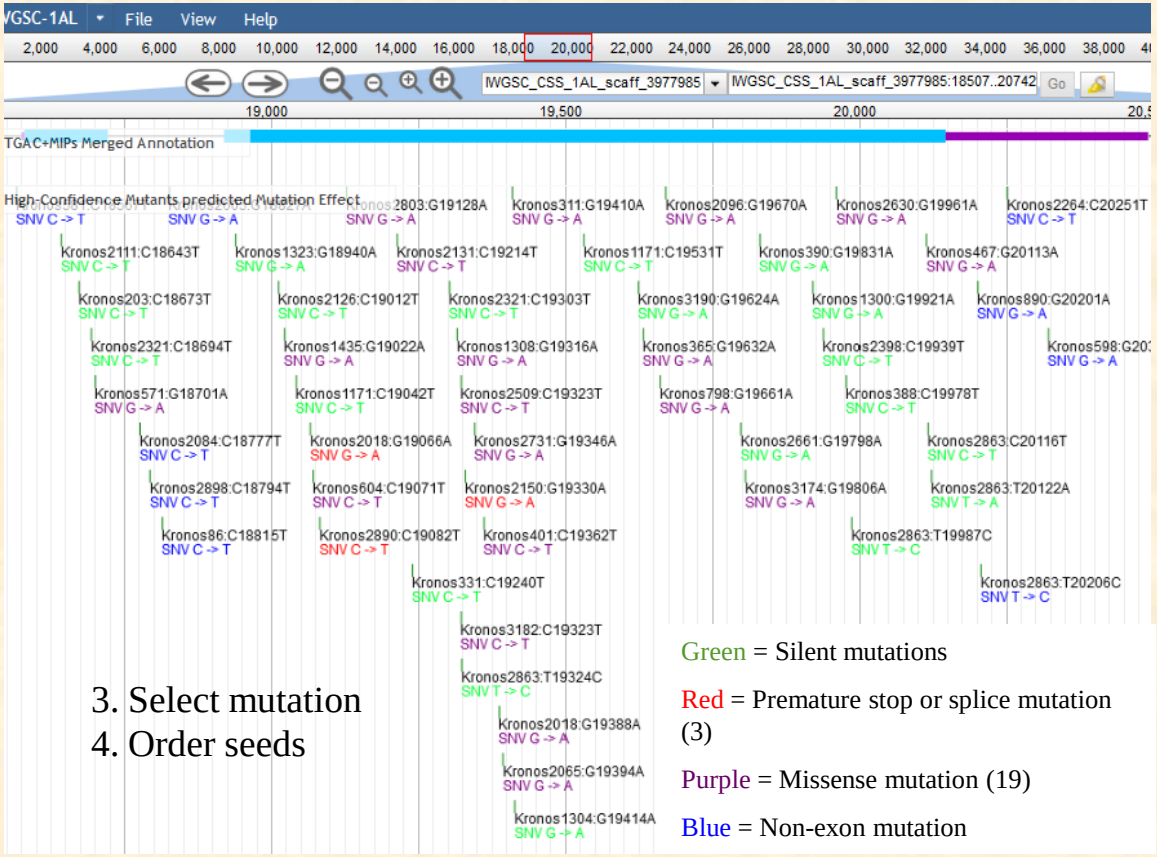


Pasta wheat (4x)

35 mutations/kb

Bread wheat (6x)

39 mutations/kb



3. Select mutation
4. Order seeds

Green = Silent mutations

Red = Premature stop or splice mutation (3)

Purple = Missense mutation (19)

Blue = Non-exon mutation

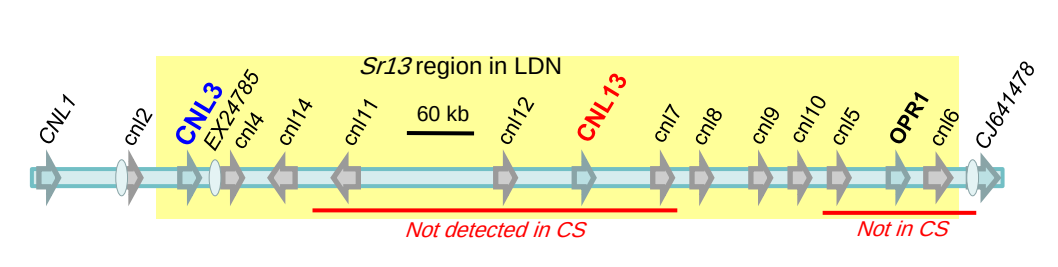
Initial impact

- >3200 individual mutant seeds and 7 complete copies of the populations already distributed.
- PNAS manuscript >20,000 downloads (>98th percentile).
- Central tool in a new 5 year IWYP-USDA project including 20 wheat programs in the USA (led by UCD).

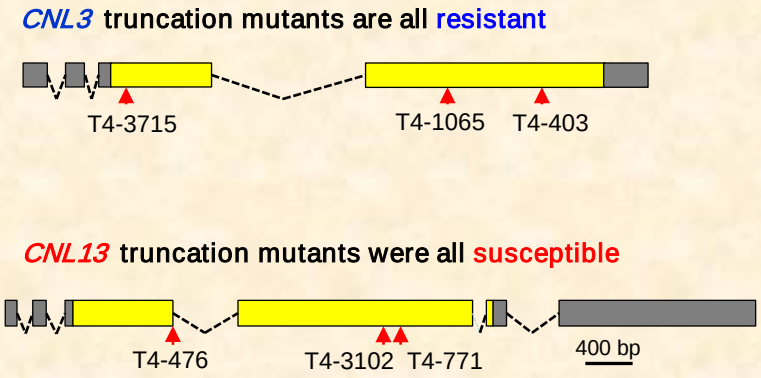
Applications of the sequenced mutant populations

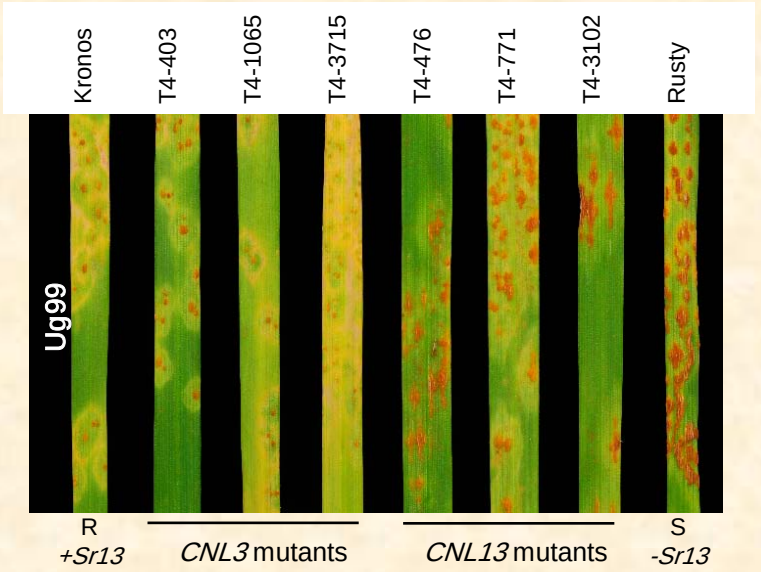
1. Candidate gene validation

- We mapped the Ug99 resistance gene *Sr13* to a 900 kb region with two NLR genes (6000 F_2)



- We identified three independent truncations in CNL3 and three in CNL13



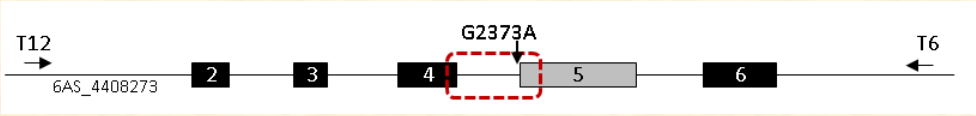


CNL13 is necessary for resistance to Ug99

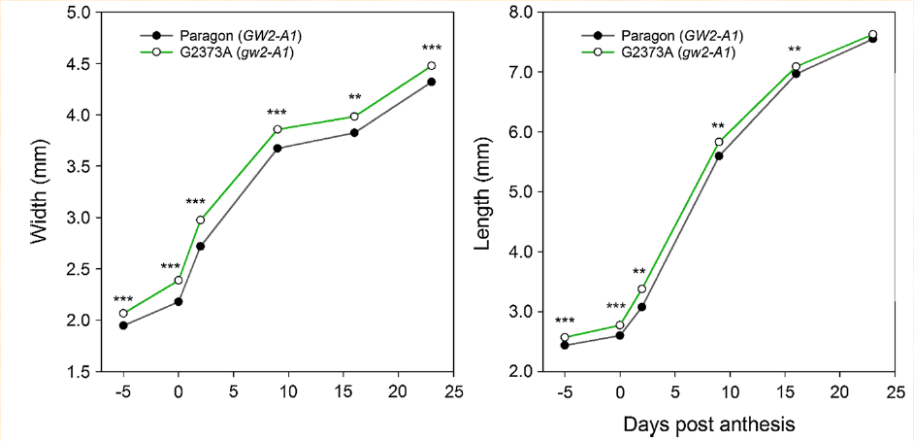
PNAS 2017. *In press.*

Applications of the sequenced mutant populations

2. **Wheat breeding:** mutations of negative regulators of seed size



Gw2 encodes a RING-type E3 ubiquitin ligase and functions as a negative regulator of grain size

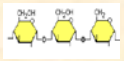


- Splice site mutations in negative regulator of seed growth ***gw2-A1***
- Average **6.6% increase in kernel weight**
- Validated in 13 greenhouse and field experiments in tetraploid and hexaploid wheat

  Theor. Appl. Genet. 2016. 129:1099–1112
- *gw2-A1* + *gw2-D1*= **9 % increase in kernel weight in common wheat**
- *gw2-A1* + *gw2-B1*= **9 % increase in kernel weight in pasta wheat**

  Unpublished

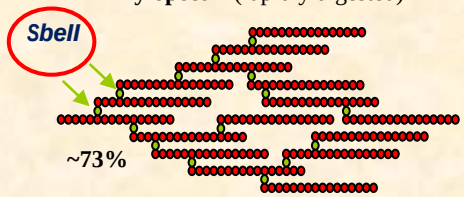
Increasing dietary fiber in wheat



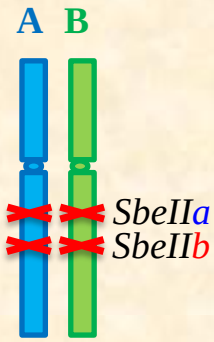
Amylose (resistant starch)

~27%

Amylopectin (rapidly digested)

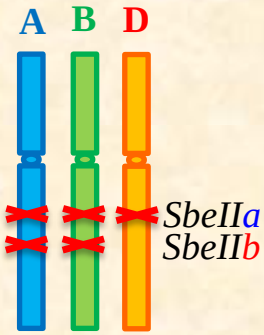


4 mutations



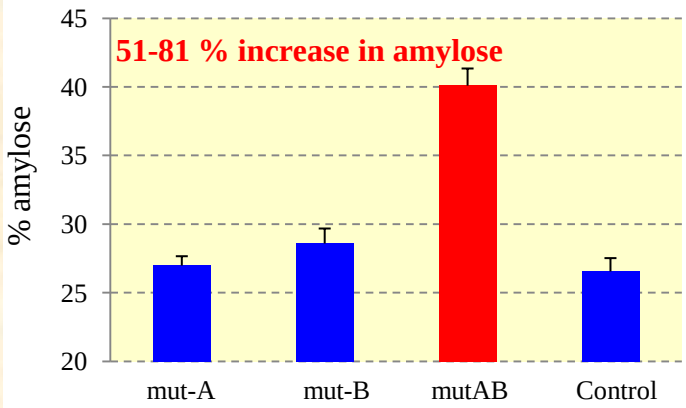
Crop Sci. 2012
J. Pl. Reg. 2015

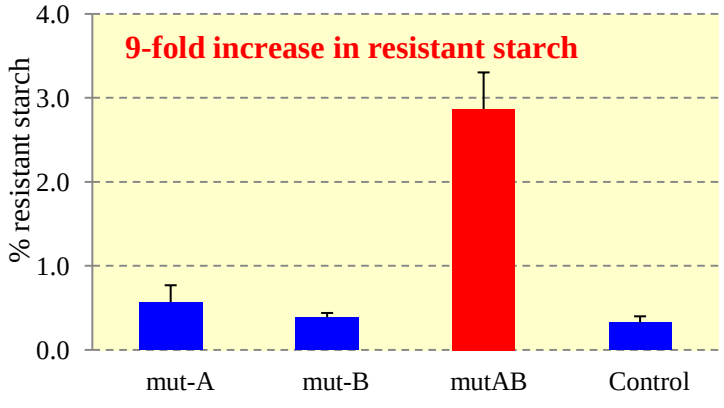
5 mutations



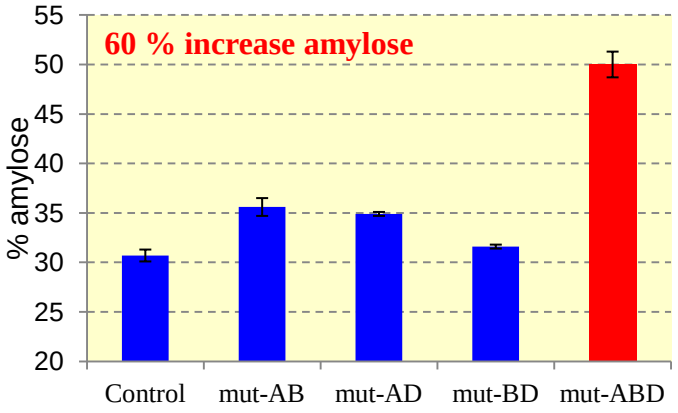
J. Pl. Reg. 2016
J. Cer. Sci. 2017

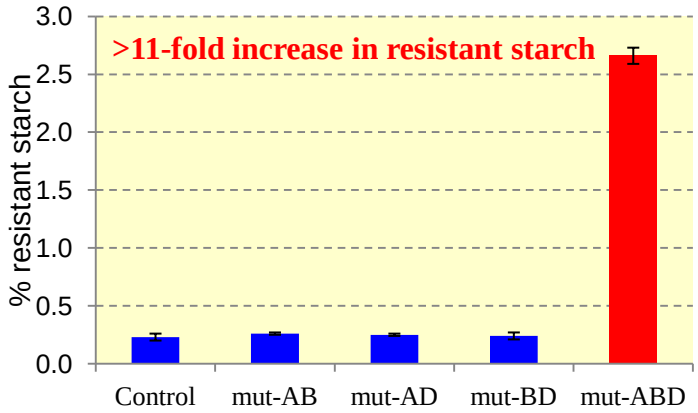
***sbeIIa* + *sbeIIb* mutants in tetraploid wheat Kronos**





***sbeIIa* + *sbeIIb* mutants in hexaploid wheat Lassik**





- Wheat seeds combining all mutations have been distributed worldwide
- We are currently releasing one pasta and two bread wheats with high resistant starch (PVP in progress)

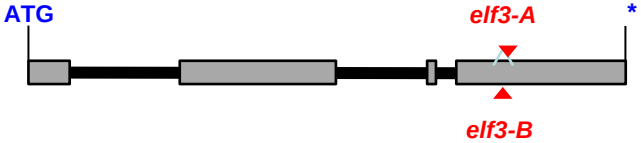
Adjusting flowering for specific environments

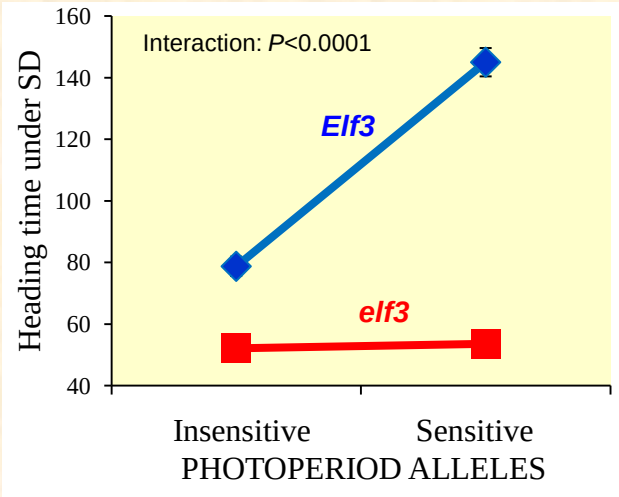
- When the duration of the day extends beyond 20 hs…
- It may be no longer useful to have a biological clock!

- Barley varieties with loss-of-function mutations in clock gene ***elf3*** are well adapted to high latitudes

Funct. Integrative Genomics (2016)

Loss-of-function mutations in wheat *Elf3* (4x)



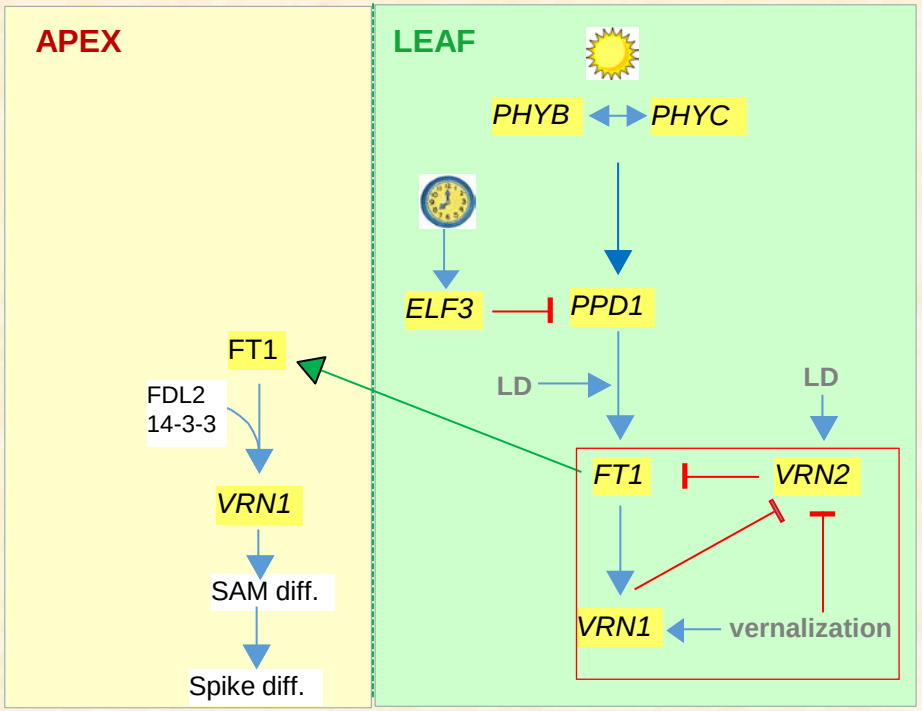


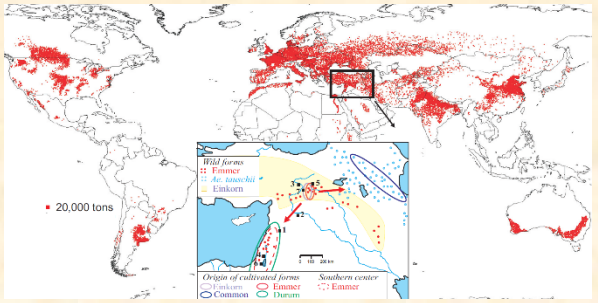
elf3 mutants in one wheat genome have limited effect on flowering.

***elf3*-null** mutants flower very early under short and long days, and in both photoperiod sensitive and insensitive wheats. We have sent *elf3*-null mutants to Finland for evaluation…

Applications of the sequenced mutant populations

Dissecting complex pathways





Natural variation in vernalization and photoperiod genes has been essential for wheat adaptation to a broad set of environments

Vernalization

VRN2: Triple *vrn2*-null mutants eliminate vernalization requirement in hexaploid wheat. TAG (2016)

VRN1: *vrn1*-null mutants showed that *VRN1* is not essential for flowering and repressor *VRN2* in spring. PLoS Genetics (2012)

VRN4: Gene cloning revealed a duplicated *VRN1* with a new allele affecting *GRP2* binding in first intron PNAS (2015)

Photoperiod

PHYC & PHYB: Both genes were required for the light activation of *PPD1* and the photoperiodic response PNAS (2014) BMC Pl. Biol. (2016)

PPD1 is essential for night break flowering acceleration, but under LD there is a PPD1-independent photoperiod pathway. Plant Phys. (2017)

ELF3 acts as a negative regulator for *PPD1* and is esential for the photoperiodic response. Funct. Integ. Genom. (2016)

FT1 promotes GA biosynthesis in the apex, which in combination with *VRN1* is essential for normal spike development. Plant Physiology (2013)

Comparison of sequenced mutant populations and CRISPR

Comparison between sequenced mutant populations and gene editing

Features	Sequenced mutant population	Gene editing
Ease of getting started	Mutations are searchable online ; immediate access to mutant seed	Requires additional time for construct design and optimization; delivery into wheat is dependent on access to technology; relatively high cost
Achieving specificity	Specific for C to T and G to A transitions; local sequence-dependent bias affects the probability that C/G positions will be mutated	Dependent on presence of PAM (5′-NGG-3′); new Cas9 specificities have been published ; new nucleases [Cpf1 (113)] have a different range of PAMs
Off-target effects	Thousands of mutations outside gene of interest with many potential deleterious mutations	Very specific with more limited off-target effects
Developing triple mutants	Mutants in individual homoeologs can be combined through traditional crossing and marker-assisted selection	Triple mutants in first generation not likely (approximately 0.5%); requires crossing of single homoeologs
Range of varieties	Original mutants restricted to sequenced populations; can be transferred to locally relevant germplasm by crossing	Dependent on transformation efficiency of variety; requires crossing to locally relevant germplasm to deploy in agriculture
Use in breeding	Currently deployed and not subject to regulation	Nontransgenic classification is still uncertain in many countries; may be problematic for globally traded crops

Base editing: fusion of “dead” CRISPR/Cas9 and a cytidine deaminase enzyme that retain the ability to be programmed with a guide RNA and mediates the conversion of cytidine to uridine, generate C→T (or G→A) substitution

Sequenced mutant populations and Cas9-mediated gene editing technologies provide complementary approaches to generate and utilize induced variation

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General conclusions

1. In the young polyploid wheat species, redundancy among homoeologs usually mask the effect of recessive mutations.
2. Combination of mutants from the different homoeologs is required to reveal that hidden variation
3. Sequenced TILLING populations provide a fast way to identify required mutations and provide a powerful tool to dissect complex developmental pathways.
4. Information about the relative effect of individual homoeologs is useful for wheat breeders to modulate the responses.
5. There are direct applications of the mutants to wheat breeding (e.g. *gw2*)
6. TILLING mutant populations generate a high amount of novel variation

The wheat sequenced mutant populations are publicly available and are expanding the way we do wheat functional genetic analyses

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Sequenced tilling populations
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Grain Protein content gene
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Disease resistance
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MAPS pipeline to identify mutations

- Polymorphism detection MAPS pipeline from Luca Comai Lab (we adapted it to polyploid wheat)
  - http://comailab.genomecenter.ucdavis.edu/index.php/MAPS
  - Published in ***Plant Cell*** 2014, 26:1382–1397

Noise:

Varietal SNP

Paralog/Homeolog SNP

Sequencing/PCR error

Signal:

EMS Mutation