

The Fascinating World of Diploid Alfalfa

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Brian Irish, USDA-ARS-NPGS



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AFRI Award #2023-67013-39617 to EC Brummer and
JG Monroe.





Context
Phenomes
Genomes
Prospects

ORIGINAL ARTICLE

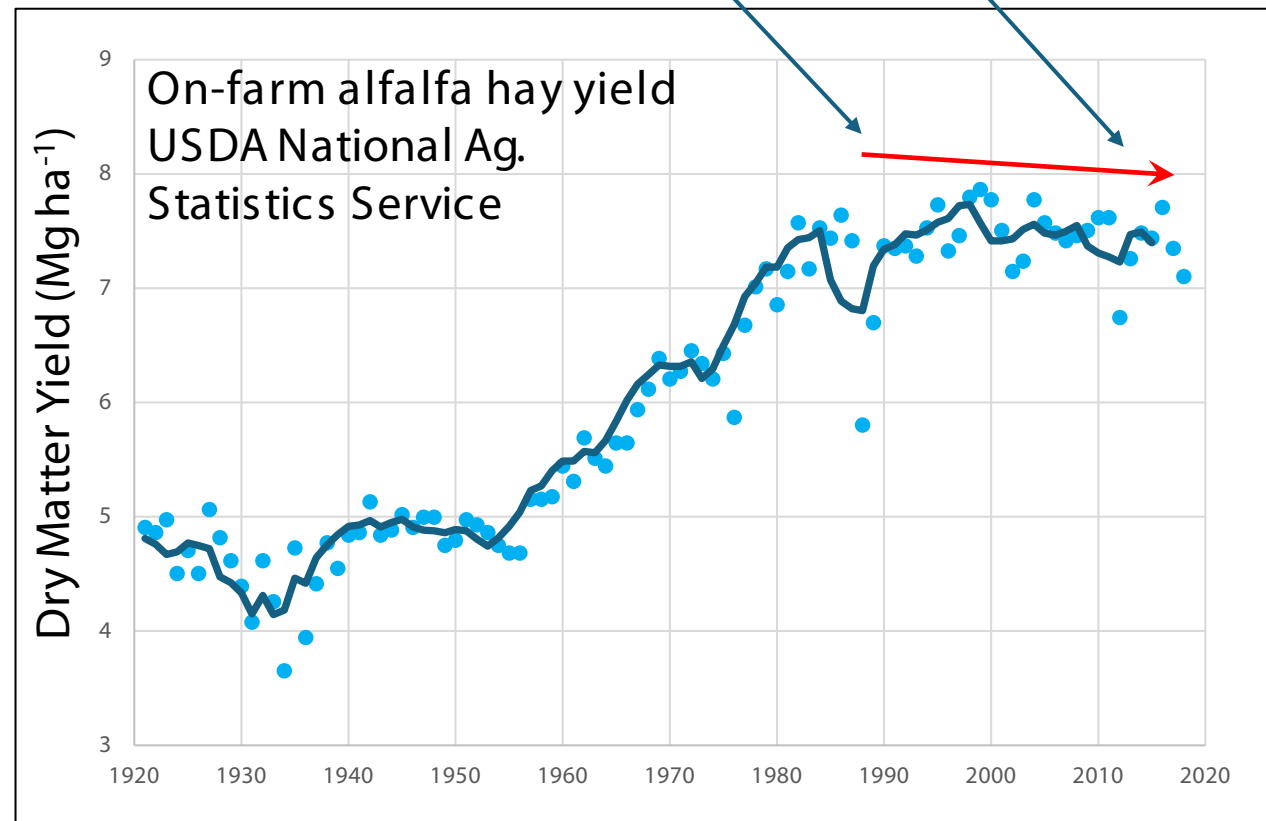
US alfalfa variety trials show breeding progress for yield, broad adaptability of commercial varieties, and widespread genotype-by-trial variation

Neal Tilhou ✉, Heathcliffe Riday

First published: 23 September 2025 | <https://doi.org/10.1002/csc2.70157> | [VIEW METRICS](#)

Why aren't variety trial yield improvements carried over to on-farm yield (as they are in maize and other crops)?

[T]here was breeding progress for biomass yield (+0.63% per year; $p = 9.0 \times 10^{-78}$) in alfalfa between 1988 and 2017.





THE BREEDING OF CROP IDEOTYPES

C. M. DONALD

Waite Agricultural Research Institute,
The University of Adelaide, South Australia

Received 17 November, 1967

HIGH HOPES FOR SHORT CORN

Plants bred or engineered to be short can stand up better to windstorms. They could also boost yields and benefit the environment *By Erik Stokstad*



Tall conventional plant

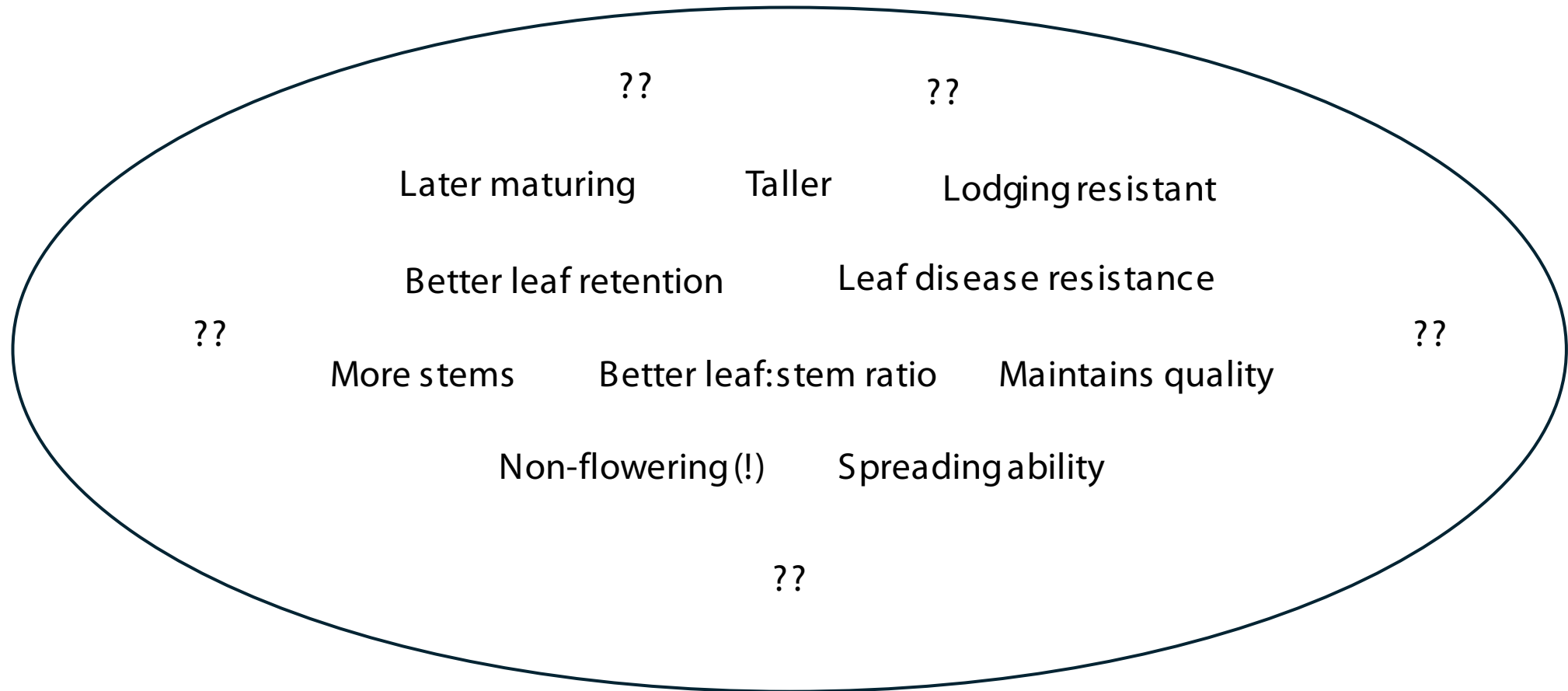


**Improved high-yielding
plant**



**Low-tillering ideotype
(new plant type)**

What is the ideotype of New Plant Type Alfalfa?



Diploid germplasm has abundant phenotypic variation for genetic mapping

Tulelake, CA



Why Diploid Alfalfa

If we want to understand morphological, phenological, developmental traits in alfalfa, diploids are a lot easier

$AA \times aa$
 $\downarrow$
 Aa
 $\downarrow$
 $\frac{1}{4} AA : \frac{1}{2} Aa : \frac{1}{4} aa$

$AAAA \times aaaa$
 $\downarrow$
 $AAaa$
 $\downarrow$
 $\frac{1}{36} AAAA : \frac{17}{18} A--a : \frac{1}{36} aaaa$



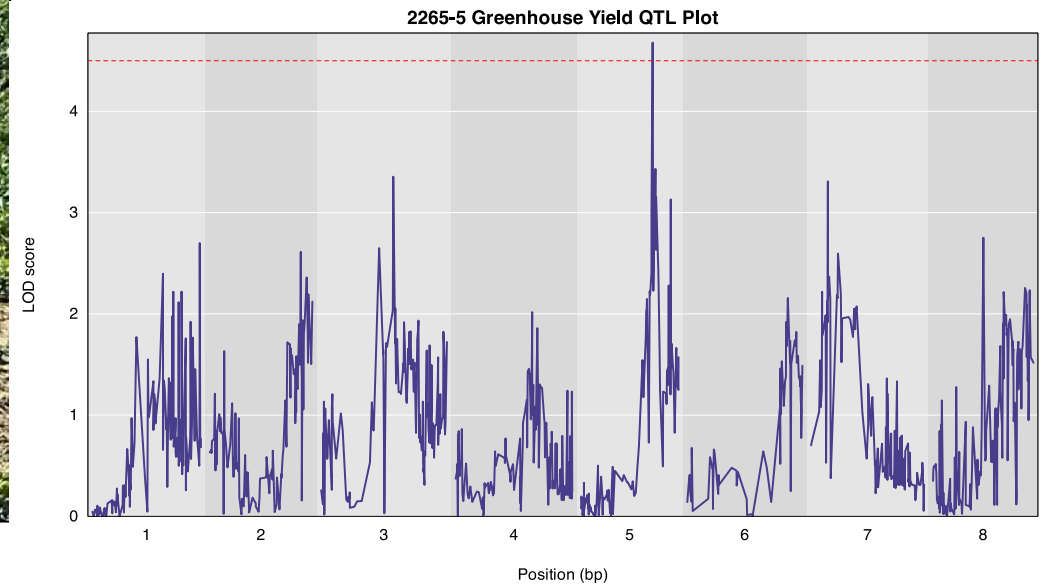
Preliminary biomass QTL mapping



2265-05 F₂ population

Significant QTL on Chrom 5 near
distorted region

(PVE = 12%)





Creeping
Roots

Ornamentals



Diploid Alfalfa Genome Sequencing

Cree King, Matt Davis, Grey Monroe

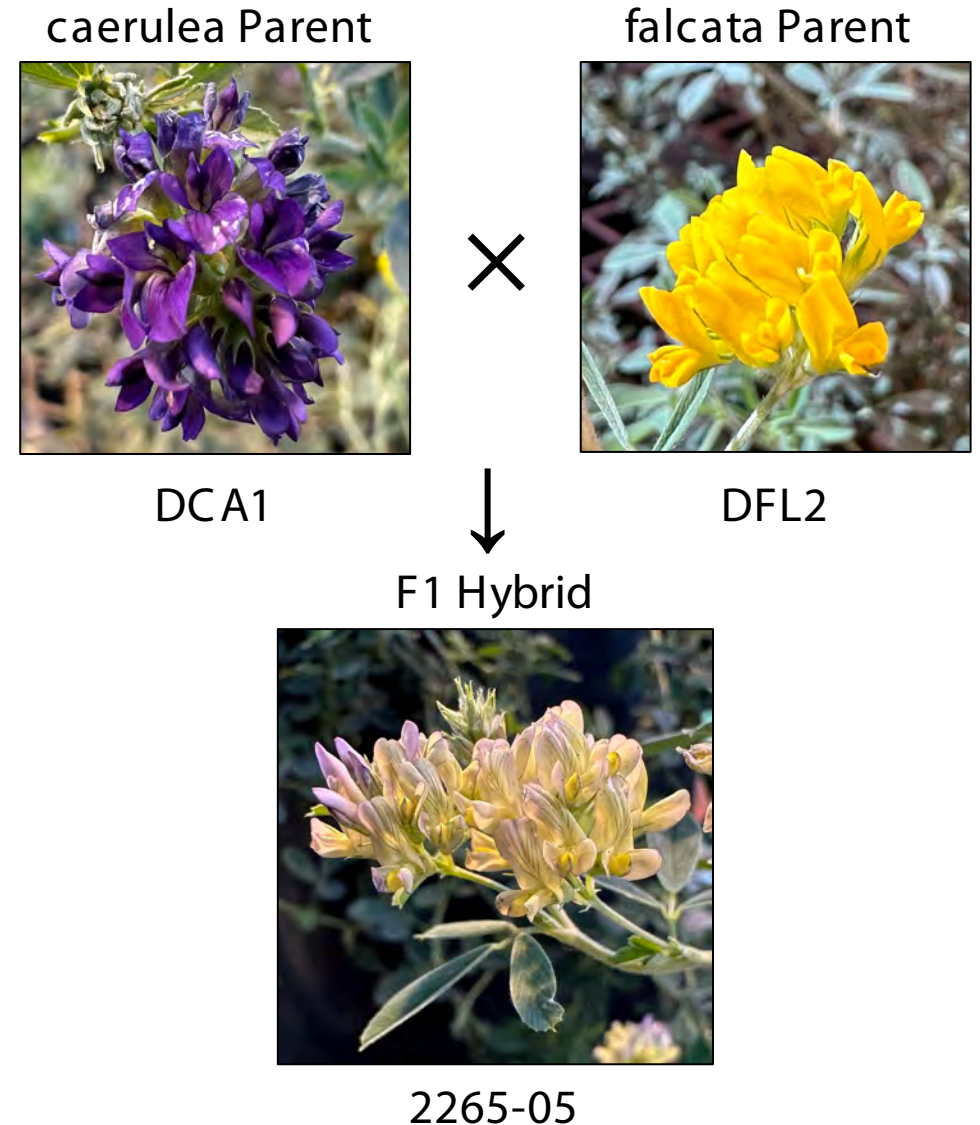
Inter subspecific cross

Medicago sativa subsp. *caerulea* × subsp. *falcata*

Sequencing all three plants with PacBio HiFi
(Revio)

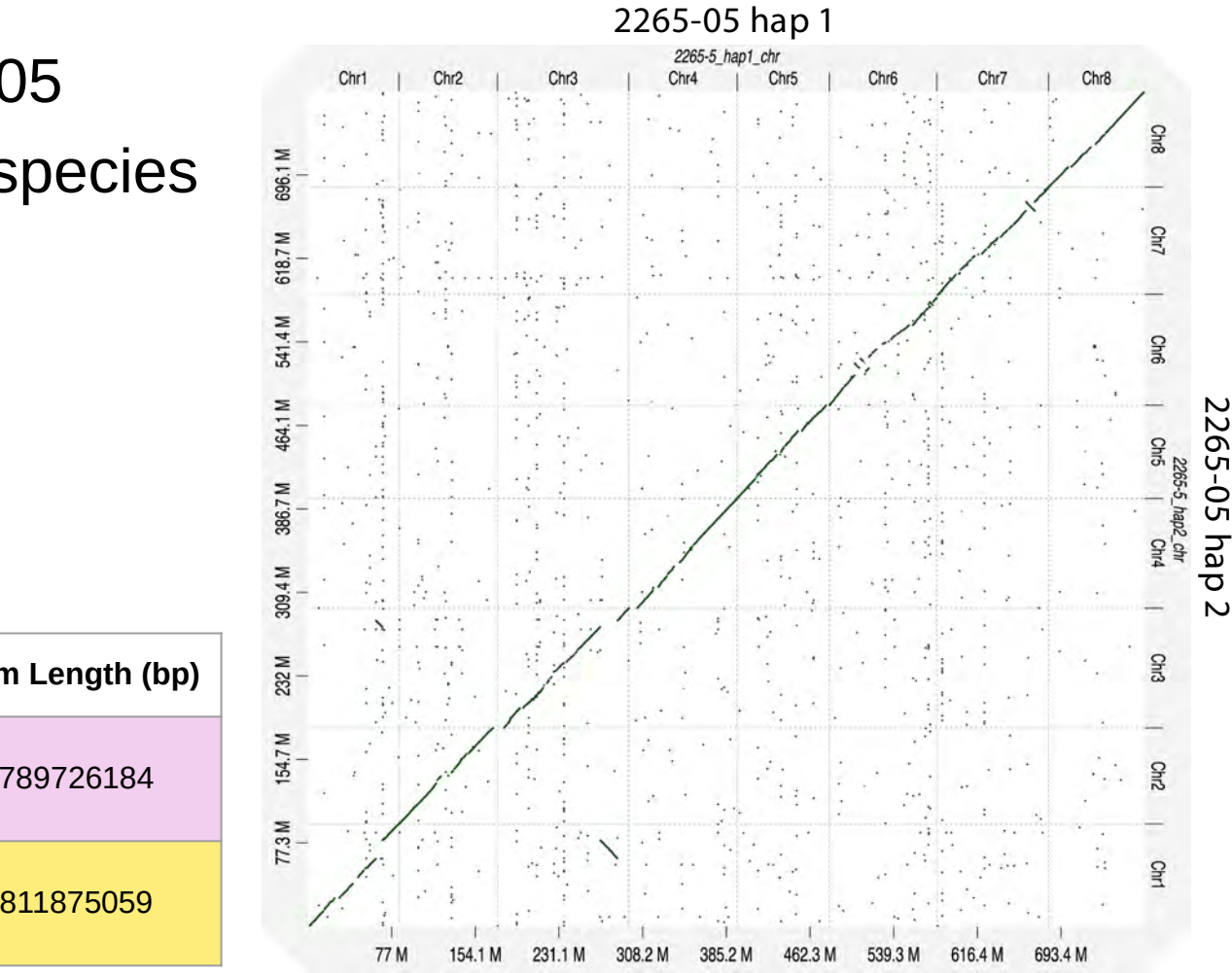
Contig assembly with HiFiasm using trio
binning to resolve haplotypes in 2265-05

Scaffolding of 2265-5 with Omni-C data
using YAHS + Juicebox



Diploid Alfalfa Genome Assembly

- 🌱 Haplotype phased assemblies of 2256-05
 - 🌱 Each haplotype represents one subspecies
 - 🌱 Switch Error rate (W) = 0.0056%
 - 🌱 Hamming Error rate (H) = 0.011%
 - 🌱 Haplotypes are largely colinear
 - 🌱 Read N50 of 16,458 bp



Assembly	Num Seqs	Min Length (bp)	Contig N50 (bp)	Sum Length (bp)
2265-5 Hap 1 (DCA1)	355	13916	21296290	789726184
2265-5 Hap 2 (DFL2)	775	5259	26939162	811875059

Gene Annotation of 2265-05

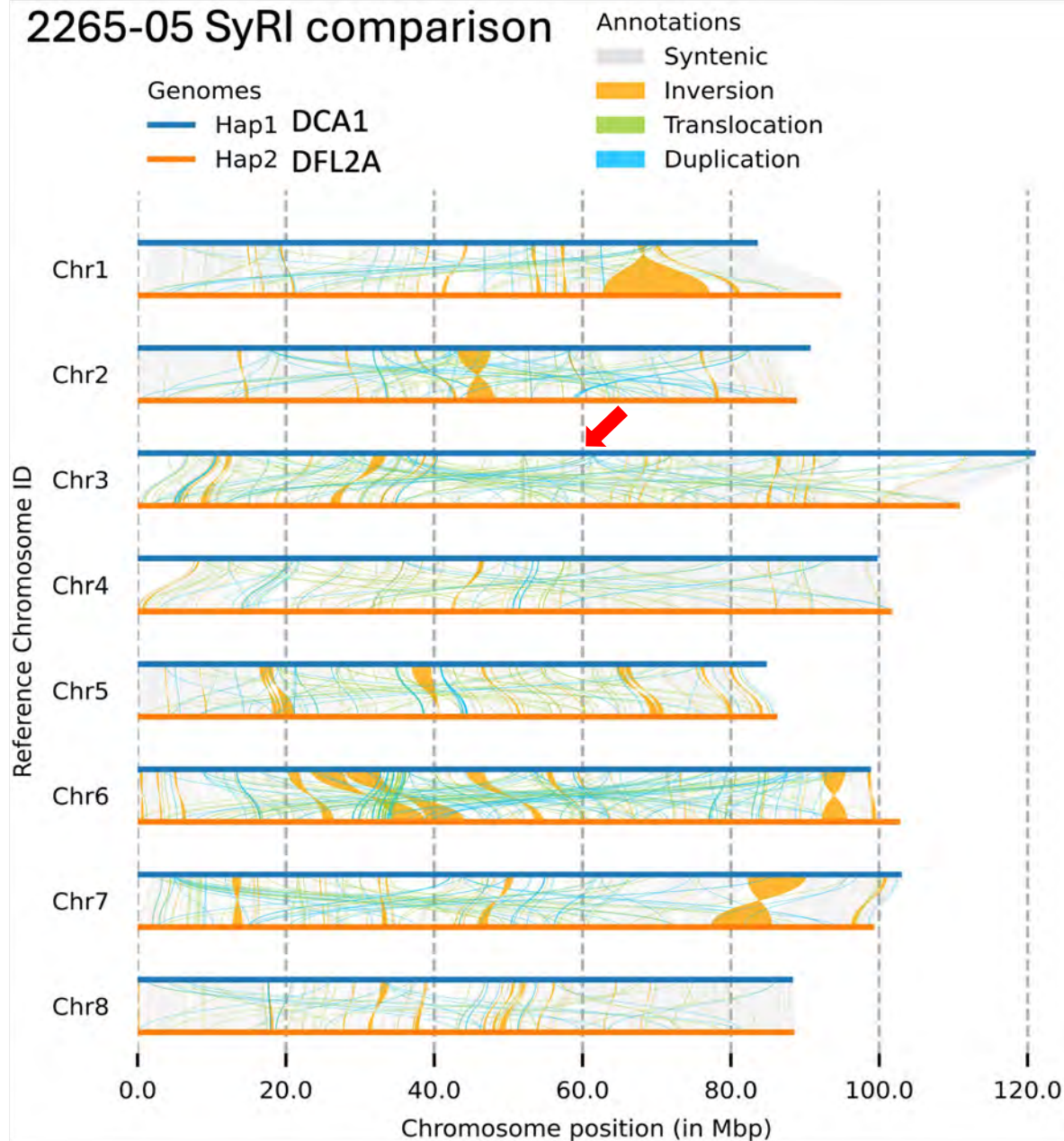
Evidence based gene annotation using PacBio RNA Iso-seq

Circadian collection of tissue (4 time points, including leaf, stem, flower, and root tissues from 2265-05 hybrid and both parents (DCA1, DFL2)

BUSCO Scores for 2265-05 RNA Iso-seq data:

Assembly	C% (Complete)	S% (Single-copy)	D% (Duplicated)	F% (Fragmented)	M% (Missing)	# Identified Genes
Hap 1 (DCA1)	97.2	91.2	6.0	1.0	1.8	55608
Hap 2 (DFL2)	97.7	92.0	5.7	0.9	1.4	57094

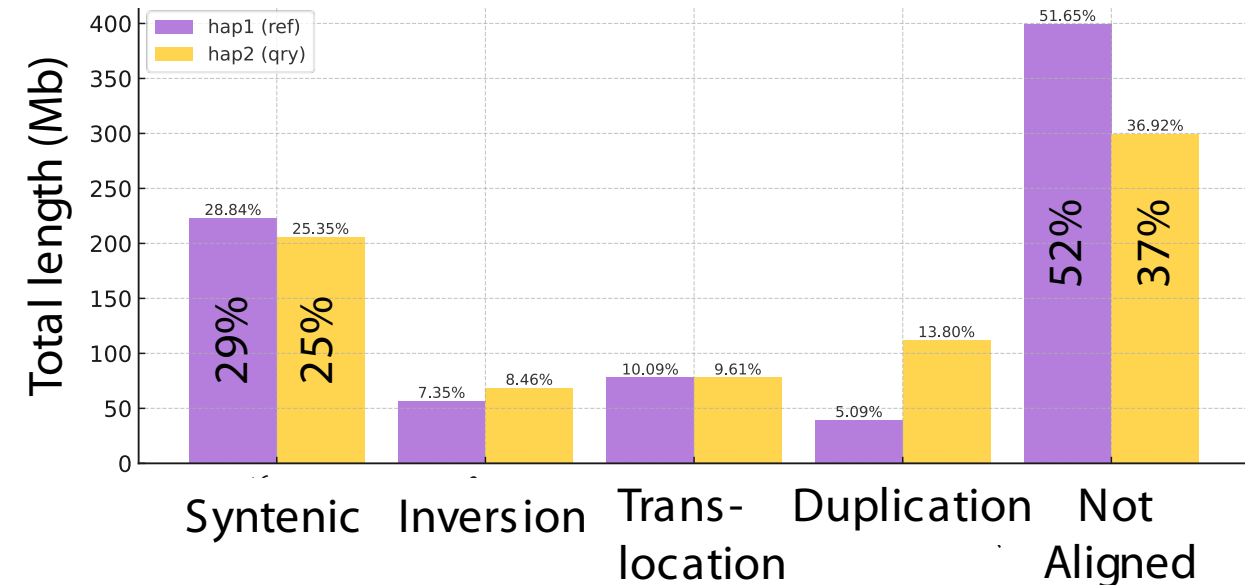
2265-05 SyRI comparison



Structural Variation between Subspecies in 2265-05

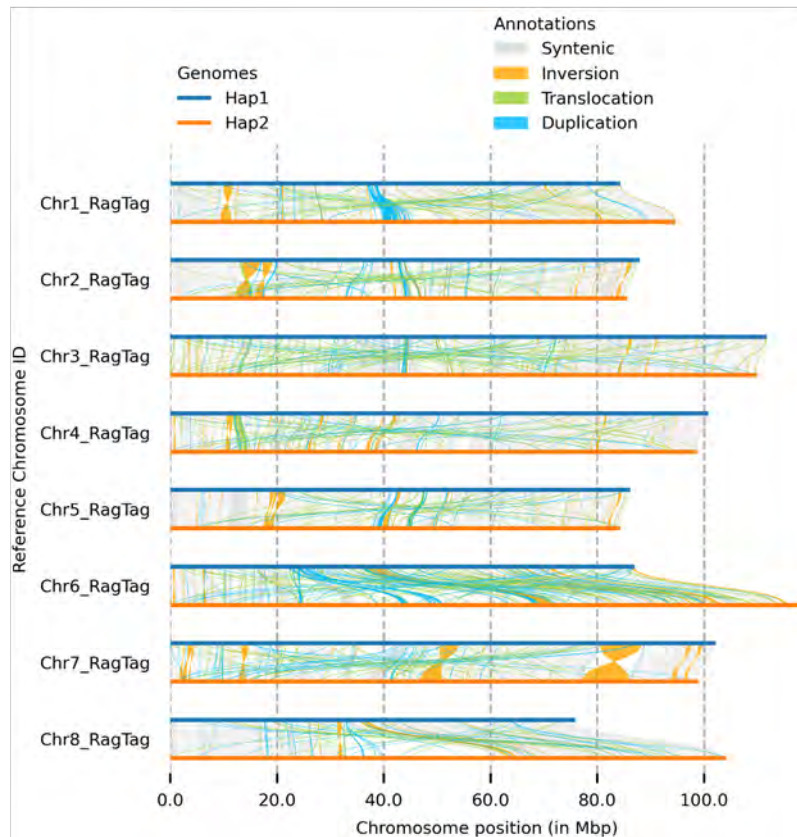
- 🌱 SyRI: Synteny and Rearrangement Identifier
- 🌱 Large number of rearrangements between the two homologues
- 🌱 Possible transposon activity (red arrow)

SyRI Structural Variation Summary

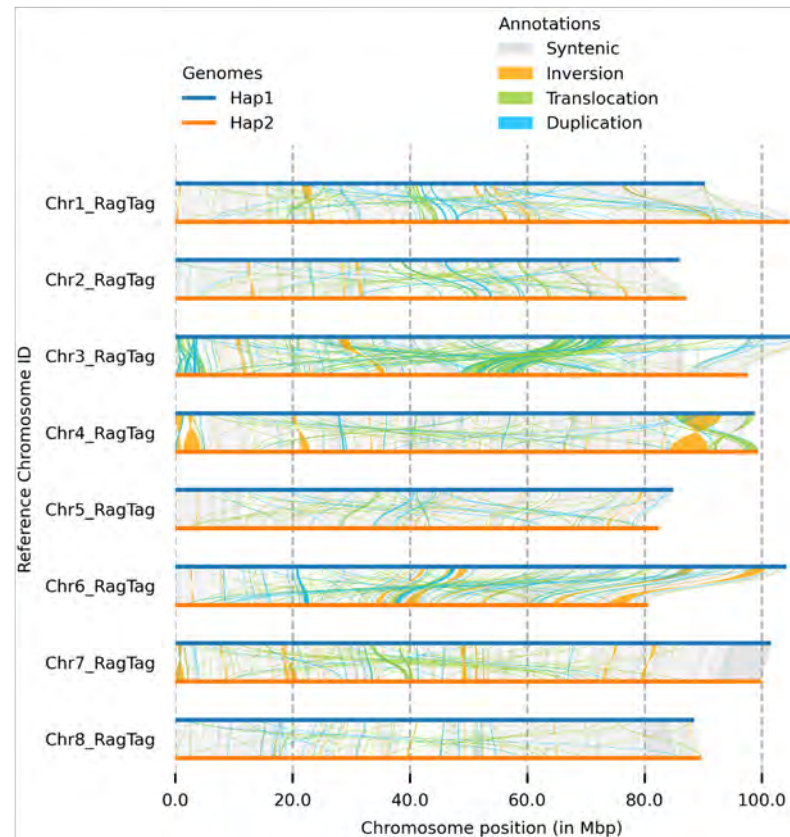


Intra-subspecies Structural Variation

DFL2A
falcata



DCA1
caerulea



- Large number of changes *within* haplotypes of the same subspecies
- Similar to 2265-05
- Alfalfa genome seems to be very dynamic

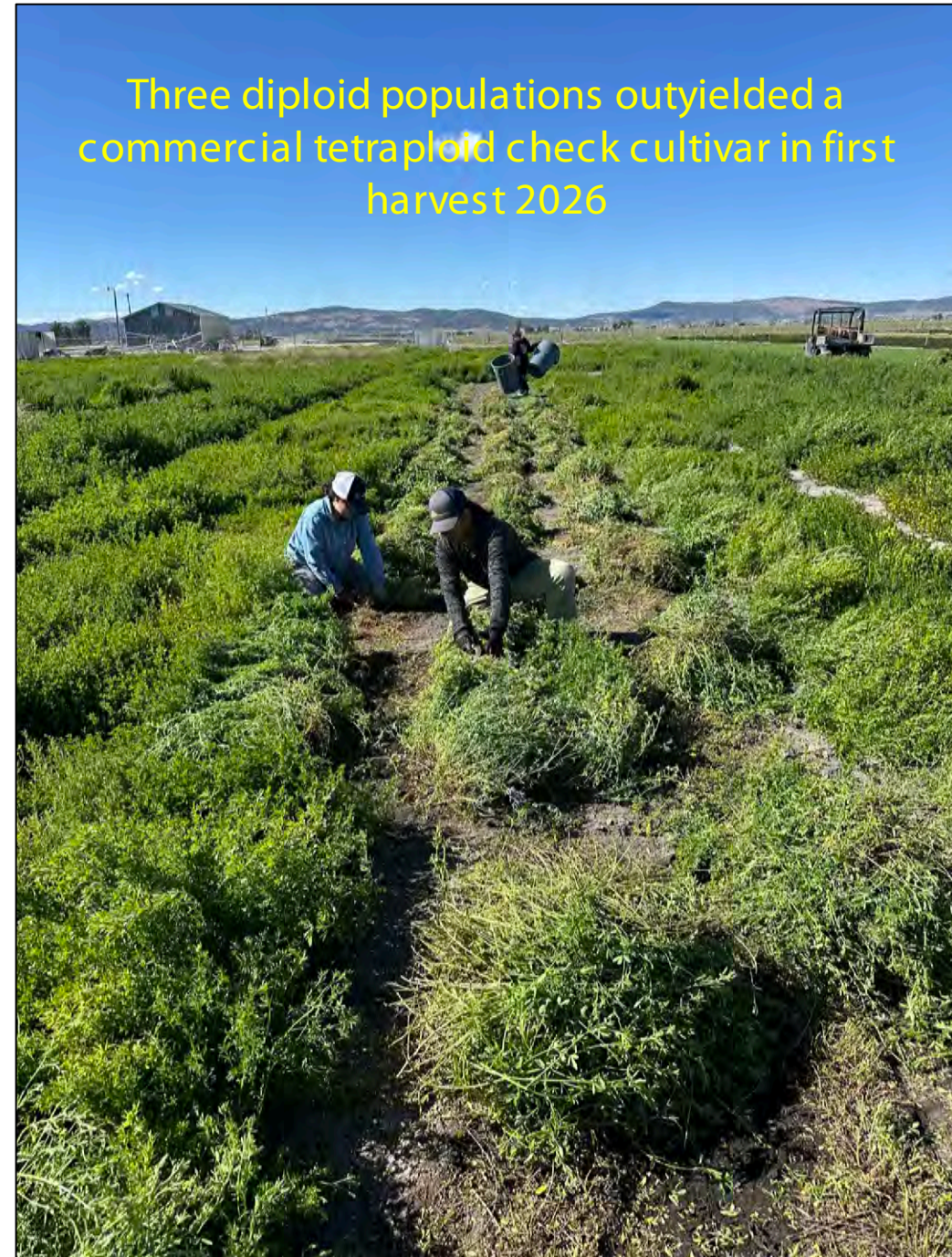
SyRI comparisons between haplotypes of parents

Reinventing Potato as a Diploid Inbred Line–Based Crop

Shelley H. Jansky,* Amy O. Charkowski, David S. Douches, Gabe Gusmini, Craig Richael, Paul C. Bethke, David M. Spooner, Richard G. Novy, Hielke De Jong, Walter S. De Jong, John B. Bamberg, A. L. Thompson, Benoit Bizimungu, David G. Holm, Chuck R. Brown, Kathleen G. Haynes, Vidyasagar R. Sathuvalli, Richard E. Veilleux, J. Creighton Miller, Jr., Jim M. Bradeen, and Jiming Jiang

Crop Sci. 56:1412–1422 (2016). doi: 10.2135/cropsci2015.12.0740

Three diploid populations outyielded a
commercial tetraploid check cultivar in first
harvest 2026



Prospects

Genetic basis of traits can be dissected through mapping easier in diploids

Gene editing can be done more easily in diploids

Diploids may have useful alleles for agronomically important traits



A close-up, high-angle photograph of a dense field of flowers. The image is filled with numerous small, bright yellow flowers and clusters of small, vibrant purple flowers. The flowers are interspersed with lush green foliage, including thin stems and small, pinnate leaves. The overall scene is a vibrant, natural display of flora.

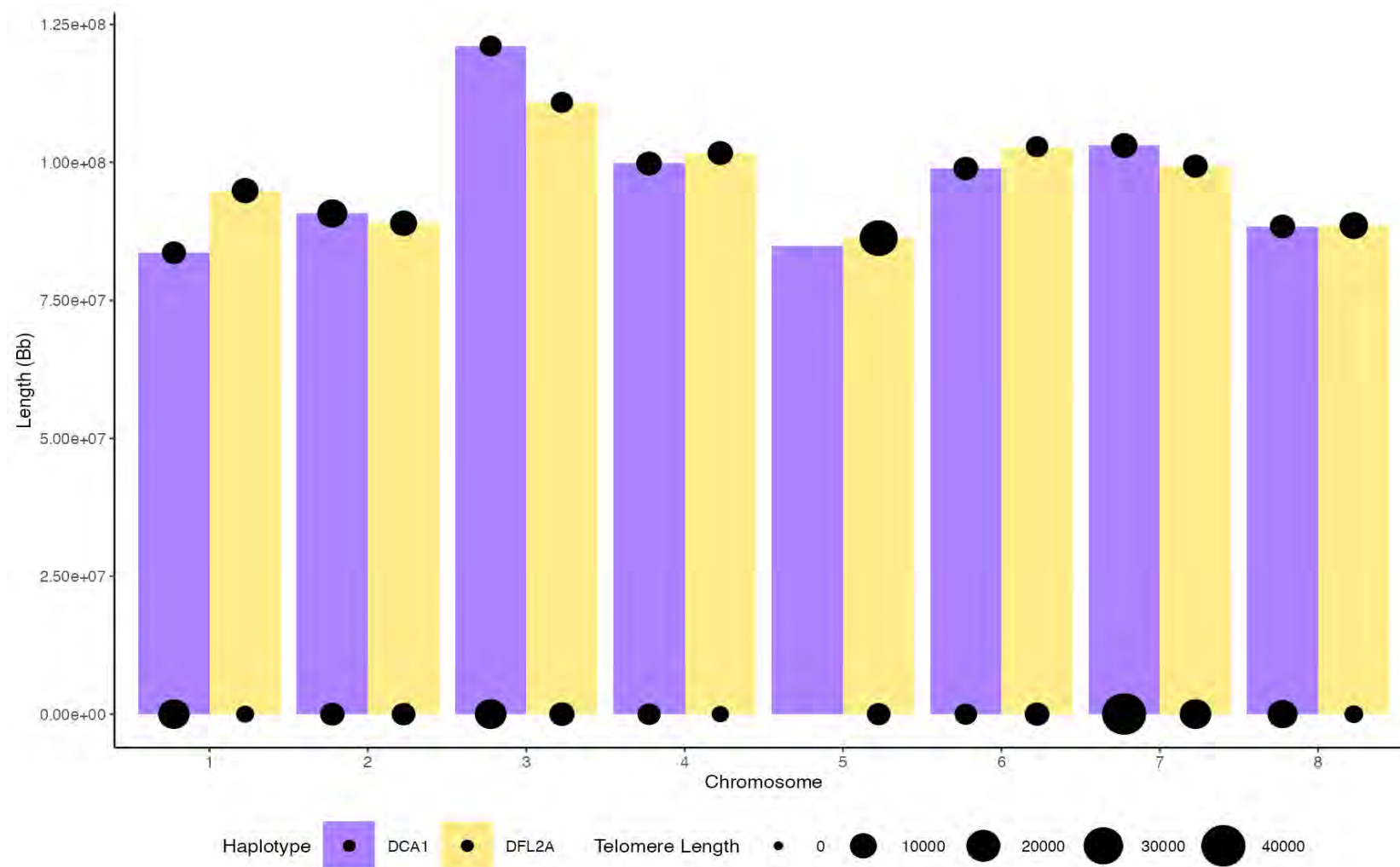
THANK YOU!

Diploid Alfalfa Genome Assembly of 2265-05

HiFi sequencing
produced high quality
long reads

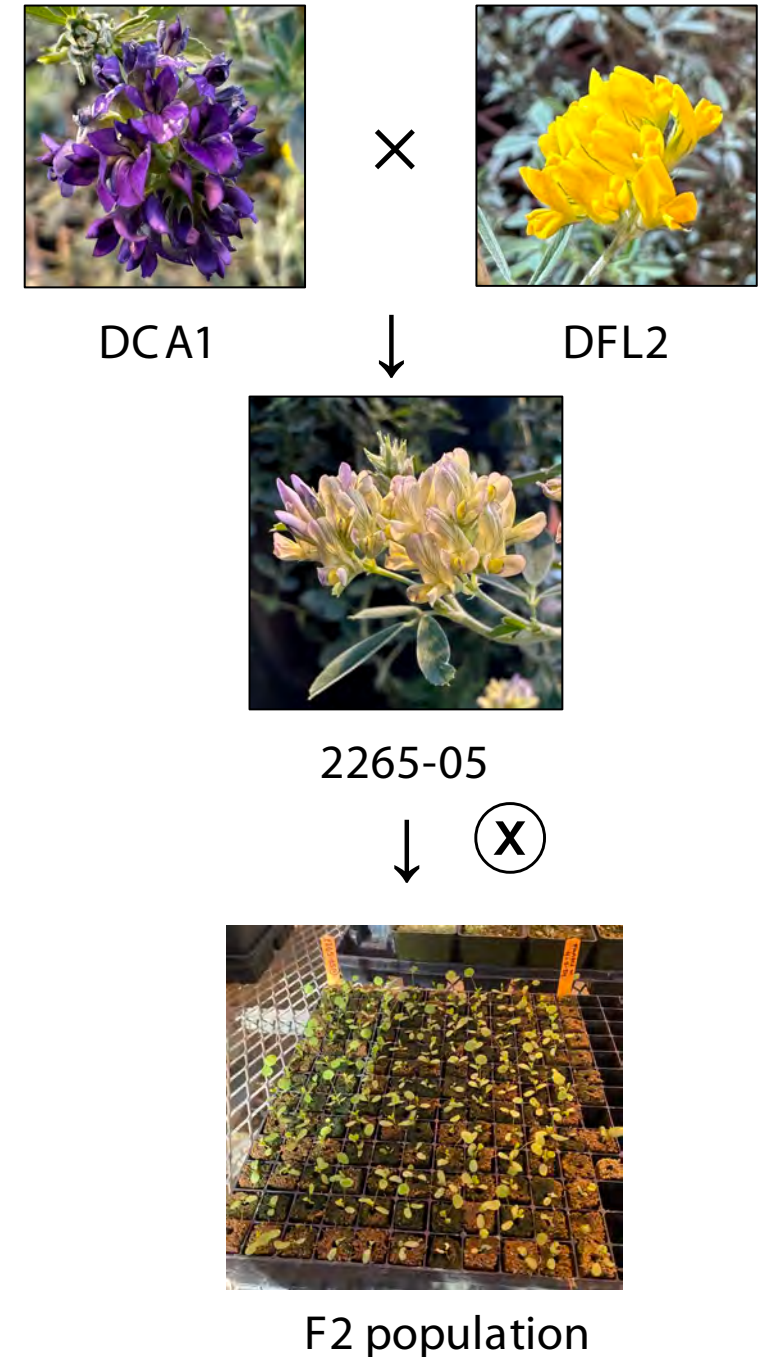
Sequencing depth of
135x

Contiguous
sequences telomere
to telomere



Segregation Distortion

- 🌱 DArTag genotyping of F_2 population of >400 individuals from selfing 2265-05
- 🌱 Aligned to 2265-05 hap 2 to visualize regions exhibiting segregation distortion
- 🌱 Is distortion related to structural variation?



Segregation distortion across the genome

Expectation of any segregating marker locus in a diploid F_2 population
25% AA (black) : 50% Aa (red) : 25% aa (blue)

Excess heterozygosity on all Chromosomes except Chr. 1

