

Genomic approaches to unravel the landscape of complex traits in perennial crops

I am broadly interested in the genomic and molecular mechanisms underlying key traits in perennial and winter annual crop species that can enhance the resiliency of modern farming systems by providing ecosystem services and new economic incentives for growers. In most cases, these species are undomesticated ‘alternative crops’ and require significant improvement in order to fit into modern agroecosystems. The application of modern genomic tools to improve a new species enables *de novo* domestication over the course of years instead of millennia. In my work, I have created the genetic and genomic resources needed to drive modern breeding programs for completely uncharacterized species.

Graduate Research Accomplishments: Domestication of Field Pennycress (*Thlaspi arvense*)

Pennycress has been targeted for direct domestication due to its close relation to the model plant *Arabidopsis*, its small diploid genome, and relatively high oilseed production. The goal of the domestication program is to develop the species into a winter annual ‘cash’ cover crop that can be relay or double cropped in the corn/soybean rotation in the Midwestern US. In 2012, we began a mutation-breeding program for pennycress to develop lines with reduced seed dormancy, early flowering, low glucosinolates in the seed, high oil content, and improved oil quality. The bulk of my research was developing the necessary genomic resources for pennycress, including a transcriptome (Dorn et al, 2013) and annotated draft genome (Dorn et al, 2015). We were able to flex the immense translational power of *Arabidopsis* and apply it directly to pennycress, as the genetics of these traits had largely been elucidated in *Arabidopsis*. Using these tools, we identified allelic mutants for each of these traits in mutant populations, and developed markers to begin stacking these domestication traits. In under a decade, we will have brought pennycress from a minor weed to a new winter annual cash crop.

Postdoctoral Research Accomplishments: Genomics-driven improvement of Kernza™

Thinopyrum intermedium (common name: Intermediate Wheatgrass or IWG, commercial name: Kernza) is being developed as a new commercially viable crop by The Land Institute and the Forever Green Initiative. Kernza provides essential ecosystem services by stabilizing soils with its deep (>3 meters) root structure and reducing nutrient runoff, all while producing >1,000 kg/ha of grain that is similar to wheat in food products. Over the past decade, Kernza has been dramatically improved with traditional breeding, focusing on traits relating to seed size/yield and early maturity. There have been limited molecular breeding and genomic tools for Kernza due to its large allohexaploid genome. In order to apply modern genomic breeding techniques to Kernza, we have developed an annotated, reference-quality genome assembly as a part of our DOE-JGI project, on which I was a Co-PI. This assembly is a vital resource for ongoing breeding and molecular studies to improve Kernza as a perennial grain crop. The genome sequence is currently being used for GWAS and QTL mapping studies for key domestication traits. I was also able to clarify the diploid progenitors for this allohexaploid species using genotyping-by-sequencing and transcriptomic approaches. As this species is a tertiary source for disease resistance genes in wheat breeding programs, I have also used the assembly to develop a pipeline for identifying wheat-breeding lines harboring alien translocations that contain resistance genes (Dorn and Gao et al., unpublished).

Future research program: Genomics-guided improvement of perennial grain crops

My group will focus on applying modern genomic and breeding tools to Kernza and perennial wheat to characterize the molecular and genomic mechanisms governing traits of interest. We will serve as a hub for basic research in these crops and provide new tools and traits for deployment in applied breeding programs across the world. The lab will initially focus on the key areas of interest described below.

- 1.) Genomics and molecular genetics of key Kernza traits:** I already have several projects addressing critical agronomic/end-use traits in Kernza, including mapping and characterizing genes controlling the major domestication traits. For these traits, my collaborators and I have identified major QTLs and identified candidate genes based on information from related species. We would focus initial efforts on:
 - 1A.)** Fine mapping and cloning candidate genes using whole genome sequencing approaches.
 - 1B.)** Examining variation at these loci across breeding populations to develop direct molecular markers (ex. KASP) and conduct association mapping for these traits.
 - 1C.)** Characterizing expression profiles of candidate genes to identify putative mechanisms with RNAseq.

1D.) Functional genomics of candidate genes to confirm function, including transformation/knockout experiments in model species (*Brachypodium*) and bread wheat.

1E.) Establish a transformation system in Kernza (or a diploid progenitor) for functional genomic studies

2.) Unraveling the genomic landscape of perenniality to enhance perennial wheat breeding: There is only a nascent understanding of the mechanisms underlying the perennial growth habit. The century old goal of developing high yielding, vigorously perennial wheat (and other annual crops) has progressed slowly. As a part of our JGI project, I have already developed an RNAseq dataset focused on examining an annual/perennial species pair with sequenced genomes (the annuals: *Brachypodium distachyon* *Triticum aestivum* vs. perennials: *B. sylvaticum* and Kernza). I am also developing novel germplasm resources that will be used in conjunction with the above cross-tribe RNAseq experiment to explore the perennial regrowth trait. Briefly, using a wheat addition line containing a single chromosome (4E) from *Th. elongatum* that exhibit perennial regrowth, I am generating a recombinant panel containing independent introgressed 4E segments. We have also generated a radiation hybrid panel in this 4E addition line. With the new genetic and genomic tools in place across the plant kingdom, we are now poised to dissect the mechanisms underlying perenniality, with the goal of applying this knowledge towards truly ‘perennializing’ grain crops like wheat using the experiments described below.

2A.) Exploring the epigenetic and transcriptional regulation of vernalization and flowering gene networks using the existing JGI project RNAseq dataset and new experiments focusing on examining epigenetic reprogramming (ex. histone methylation) throughout the perennial life cycle.

2B.) Knocking in/knocking out perenniality in the above recombinant and RH 4E addition line panels. Mapping common additions/deletions through GBS or WGS should narrow in on candidate regions.

2C.) Functional characterization of candidate genes, including generating transgene constructs containing candidate genes, generating/characterizing *B. distachyon* and bread wheat transformants.

3.) Mapping sources of disease resistance from wild wheat relatives: Kernza (*Thinopyrum intermedium*) is the source of many disease resistance genes in wheat breeding programs. With the high quality Kernza genome assembly and GBS data from well-characterized wheat lines harboring *Th. intermedium* translocations, I have delineated the translocated segments of the *Th. intermedium* genome present in these wheat lines. I have characterized translocations that confer tolerance against Wheat Streak Mosaic Virus (WSM1), Barley Yellow Dwarf Virus (BDV2), and several rusts (2NS), and developed a pipeline for genotyping breeding populations for the presence/absence of these segments. To further advance this work, my group would continue my collaboration with the Poland and Friebe labs at Kansas State and perform the following experiments:

3A.) Bulk segregant analysis to map candidate genes in accessions containing each translocation.

3B.) Expression profiling of candidate mutants to profile infection response.

3C.) Generating transgenics for candidate genes and field-based testing to confirm gene function.

Potential Sources of Funding: Potential sources of funding include NSF Plant Genome Research Program, NSF Molecular and Cellular Biosciences, and the USDA AFRI, and The Perennial Agriculture Project. I currently hold postdoctoral fellowship through the USDA-NIFA program, and am allowed to bring remaining funds (~\$60,000) with me when I start as new PI.

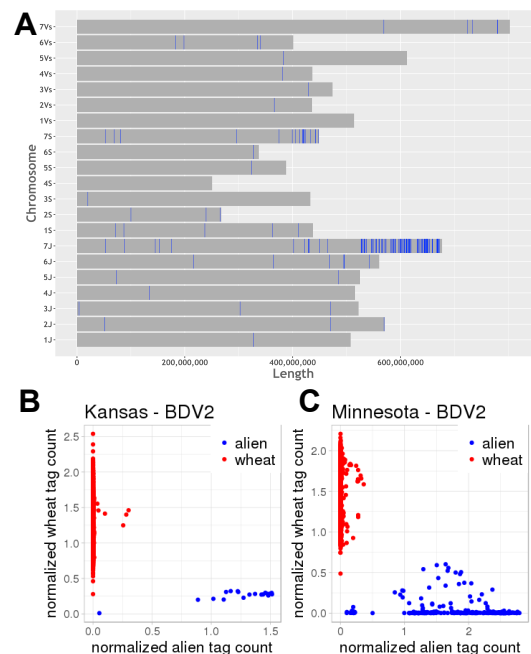


Figure 1: BDV2 translocations from *Th. intermedium* A.) *Th. intermedium* genome with BDV2 translocation specific GBS tags shown in blue – note BDV2 translocation on 7J. B-C.) Genotyping of breeding lines for 7J-BDV2 translocation in Kansas (B) and Minnesota (C) wheat breeding populations. Lines positive for containing translocation shown in blue (alien), lines negative for translocation shown in red.