

# Genomic Responses to a Century of Phenotypic Selection for Grain Protein Concentration in Maize



Christine Lucas\*; Han Zhao; Michael Zinder; Ellen Taylor; Martha Schneerman; Elizabeth Wrage; Steve Moose  
 Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, 61801

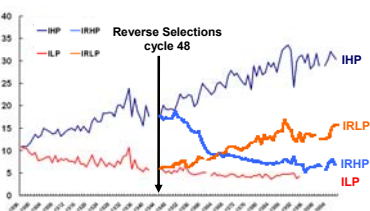
\*Contact: cjucas@uiuc.edu

## Abstract

Evolutionary changes in mRNA expression have contributed to phenotypic differences among populations. Documenting expression variation in response to artificial selection, therefore, is likely to reveal genomic targets of directed evolution. The Illinois Protein Strains (IPS) include four related maize populations (Illinois High Protein, Illinois Low Protein, Illinois Reverse High Protein, Illinois Reverse Low Protein) that have been subjected to 108 cycles of divergent recurrent selection for grain protein concentration. We have surveyed DNA sequence and expression variation in the IPS to identify genes that are likely targets of selection. Genome-scale RNA expression profiling of leaves and developing seeds from the IPS inbred lines found significant expression variation (FDR<0.05) for nearly 30% of the genes detected in all four IPS, but only 2% of these genes exhibited expression variation that was highly correlated with grain protein concentration; thus, this subset (2%) of genes represents likely targets of artificial selection. Candidate selection targets include the prolamin binding factor (*PBF*), the 19kD and 22kD  $\alpha$ -zeins (endosperm storage proteins), an endosperm-specific protease inhibitor, genes involved in nitrogen assimilation within leaves, and genes that participate in the remobilization of nitrogen from leaves to seeds. Allelic variation in DNA sequence and expression for these candidate genes is being further characterized in early and recent cycles of the selection experiment, as well as a population of 500 recombinant inbred lines derived from the cross of IHP and ILP. To date, we have obtained strong evidence that three closely-interacting genes in the key pathway modulating free asparagine levels are targets of artificial selection for plant N accumulation and grain protein concentration. One of these genes is a putative *bZIP* transcription factor that likely regulates the second gene, *asparagine synthetase3*. The third gene is an *asparaginase*, which recycles asparagine to glutamine. Collectively, analysis of the IPS populations defines genetic pathways that contribute to phenotypic differences arising from long-term artificial selection and the evolution of these pathways in response to directed breeding.

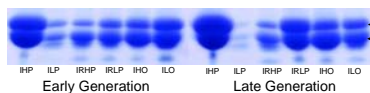
## Illinois long term selection for grain protein in maize

More than 100 generations of selection in the open-pollinated variety Burr's White have generated four populations that span the known extremes for grain protein concentration.



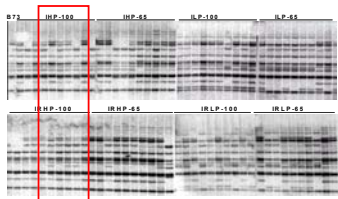
### Divergent Recurrent Selection:

- Each population was pollinated in isolation using schemes to minimize inbreeding.
- 60-120 ears were analyzed from each population per cycle.
- 20% selection index on an ear basis



The Illinois selection strains exhibit large differences in the accumulation of  $\alpha$ -zein (19kDa and 22kDa) and total N at two selection generations (~65 and ~105).

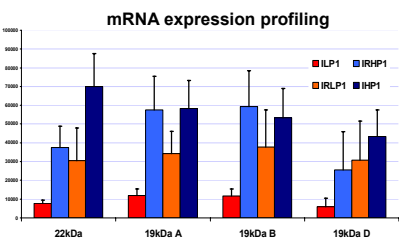
## Changes in zein gene copy number?



Allelic variation among 22-kDa  $\alpha$ -Zein genes in the Illinois Protein Strains

Allelic diversity exists, but observed patterns are not predictive of grain protein concentration. Similar results were observed for the 19-kDa zein genes. These observations further support the hypothesis that differences in 22-kDa zein accumulation are most likely regulated by *trans-acting* factors.

## Altered zein RNA expression?

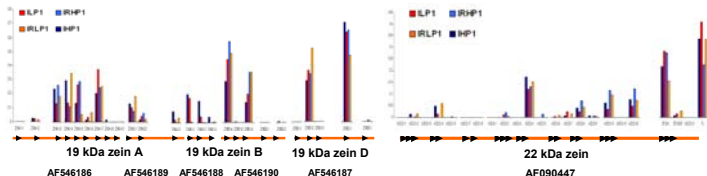


19 and 22 kDa zein gene expression patterns match the protein concentrations EXCEPT for IRHP: IRHP zein gene expression is high, but protein concentration remains low (5%).

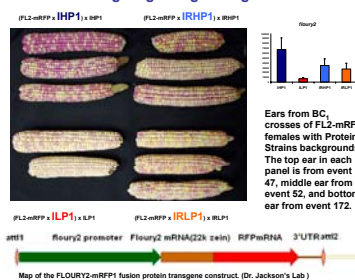
Thus, *post-transcriptional* regulation may be involved in IRHP zein accumulation.

Normalized fluorescent intensity values for oligonucleotides on the array that match over 90% expressed zein gene members of each family

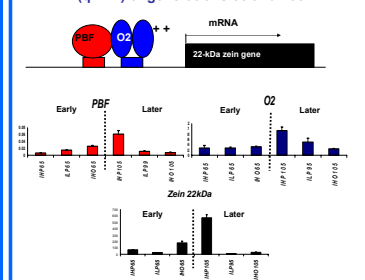
Targeted zein EST sequencing reveals that the same zein gene members are expressed in all four strains, suggesting that they are *coordinately regulated*.



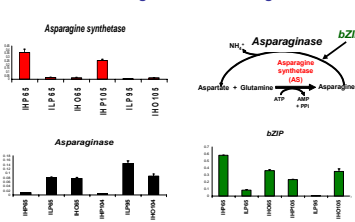
## The *floury2* RFP transgene will be a useful tool for investigating zein gene regulation.



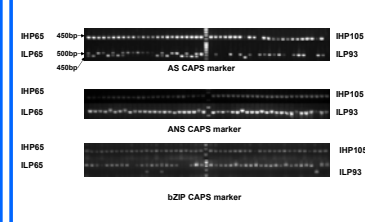
## *PBF*, *O2* and a 22kDa zein gene expression patterns (qPCR) of generations 65 and 105



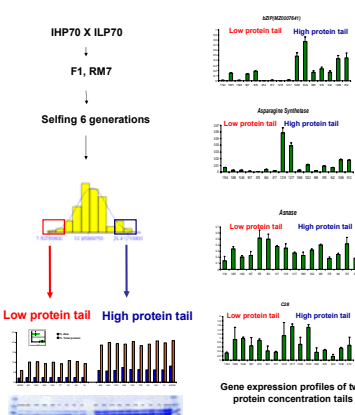
## *AS*, *ANS* and *bZIP* genes' expression patterns (qPCR) in the V14 leaves of generation 65 and generation 105



## Changes in allele frequencies in response to selection



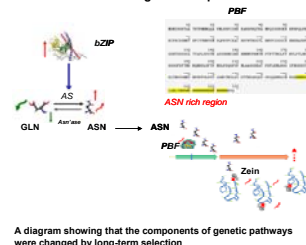
## Recombinant inbred lines derived from the cross between IHP70 and ILP70



## Summary

•Gene expression variations of leaves and seeds were observed suggesting that long term selection for high and low protein has resulted in the modification of multiple biological processes.

•Continued study of the Illinois Protein Strains promises to offer further important insights into the molecular targets of phenotypic selection and the mechanisms controlling these responses.



A diagram showing that the components of genetic pathways were changed by long-term selection

## Significance/ Future Work

We will define the molecular components and their interactions within a regulatory network connecting N status of vegetative tissues to seed protein accumulation, and assess how the activity of this network has been altered by more than a century of phenotypic selection. New insights will be gained about how breeding and selection alters quantitative variation with known epistatic interactions.

## Acknowledgements

We gratefully acknowledge the efforts and perseverance of Cyril G. Hopkins, Louie Smith, Clyde Woodworth, Earl Leng, Denton E. Alexander and Robert J. Lambert, the researchers who have served as principal investigators of the Illinois Long-Term Selection Experiment.