Population Genomics of Glyphosate-Resistant Palmer Amaranth using Genotyping-by-Sequencing (GBS)

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Outline of Talk

Background and rationale.

Methods
Investigative use of GBS scope.

Data analysis

Future

Palmer amaranth (*Amaranthus palmeri*)
- Invasive dioecious weed
- Major agricultural weed since the late 90s

Questions asked
- Has the glyphosate resistance evolved independently in the Arizona.
- Can Genotyping By Sequencing (GBS) shed light into genetic structure of the different lines of Palmer amaranth.

Samples
- Three glyphosate-resistant and five glyphosate-susceptible lines

Confirmation of glyphosate resistance
- Shikimate assay under 100μm, 500μm and 1000μm glyphosate conditions

Adapted from Dr. Kevin Bradley, University of Missouri
Confirmation of glyphosate resistance
- High shikimate accumulation in glyphosate-susceptible plants
- High EPSPS copy number in glyphosate-resistant plants

Confirmation of glyphosate resistance
Correlation of shikimate accumulation under 100 μM glyphosate conditions and genomic EPSPS copy number

Why Genotyping-by-sequencing (GBS)
- Would it work for the question asked for the study?
  - Non model plant
  - Downstream data analysis question
- Why not just EPSPS sequencing?

GBS
DNA extraction
Construct reduced representation library

GBS
1. Plate DNA & adapter pair
2. Digest DNA with ApeKI
3. Ligate adapters
4. Pool DNAs & Clean up
5. Perform PCR
6. Clean up PCR

GBS
ApeKI cut site: G CWGC
Adapted from Elshire et al., 2011

GBS
Evaluate fragment size
Run on single lane of illumina flow cell

Adapted from Elshire et al., 2011
SNP calling
- sort and count sequence tags
- filter tag counts (at least 0.4x coverage)
- remove barcodes
- produce file of DNA sequence data for each sample

Data analysis
- Align to reference genome
- UNEAK pipeline

Future work
- Account for EPSPS gene duplication
- Look into differences between males and females
- Potentially determine the origin of introduced resistant Palmer in South America

Thank you
Anita Kuepper
Colorado State University
William McCluskey
The University of Arizona
Eric Patterson
Colorado State University
Scott Nissen
Colorado State University
Scott Haley
Colorado State University
Todd Gaines
Colorado State University