Outline

1. Research program overview

2. Genomic analyses for palatability
   2.1 Genome-wide association (GWAS)
   2.2 Genomic Prediction
   2.3 GWAS signals → underlying biology
Research program focus

- Genetic control of complex traits
  - Major advances in animal genomics
  - Improve animal production efficiency & enhance animal products for human health

Healthfulness Project: development of genetic tools to improve nutritional and health value of beef.

Sustainable small ruminant production through selection for resistance to internal parasites

Genomic tools to improve meat quality traits in Angus-Brahman cattle.

Genomics of resilience in sheep to climatic stressors

Mapping QTL for breeding out of season and milk production in sheep
• Project **Goal**:  
  • Assess the natural variation in healthfulness traits  
  • Identify genetic mechanism controlling these traits  
  • Develop tools to select for:  
    ▪ More nutritious beef  
    ▪ Tasty beef  
    ▪ Improved production  

Genetic evaluation for nutrient composition of beef could result in development of **gEBV** to aid selection for:  
• favorable fatty-acid profile  
• lower levels of cholesterol and saturated fat  
• higher concentrations of minerals and vitamins
Beef Healthfulness Project

- 3 Angus herds (n = 2,285): Iowa, Oklahoma, California

• **Growth**
  - Birth, weaning, yearling, slaughter weights

• **Carcass**
  - Hot carcass weight, dressing %, ribeye area, back fat thickness, yield grade, quality grade, KPH

• **Meat Quality**
  - WBSF, Sensory panels (Juiciness, Tenderness, Connective tissue & Flavor: beef, painty/fishy, livery/metallic), TBARS

• **Nutritional value and Healthfulness**
  - Fatty acids, main FA groups (SFA, MUFA, PUFA, n3, n6), AI – Triacylglycerol, Phospholipid, Composite
  - Cholesterol, sphingolipids, creatine, creatinine, carnitine, carnosine, anserine
  - Minerals: iron, sodium, magnesium, manganese, zinc, phosphorus, potassium, calcium, copper
  - Vitamins: E, B6

All animals genotyped with the Illumina Bovine 50K SNP Bead Chip

5 generation pedigree (n = 5,907)
What do consumers want?

• **The National Beef Quality Audit**
  - Seedstock Producers, Cow-Calf Producers, Stockers/Backgrounders, Feedlot Operators, Packers, Purveyors, Retailers, & Restauranteurs
  - “*Eating satisfaction*” - only quality category for which the packers, food service buyers, and retailers are willing to pay a premium

• **NBQA Strategy Workshop**
  - Discuss the implications of research for the U.S. beef industry, provides the beef industry with a blueprint for the next five years
  - **Top 10 Quality Challenges:**
    1. Low overall uniformity & consistency of cattle, carcasses, cuts
    2. Inappropriate carcass size & weight
    3. Inadequate *tenderness* of beef
    4. Insufficient marbling
# National Beef Quality Audit

## Quality Challenges

Ranked according to priority, 1991 to 2011

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td><strong>External Fat</strong></td>
<td>Overall Uniformity</td>
<td>Overall Uniformity</td>
<td>Traceability</td>
<td>Food Safety</td>
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<td><strong>Seam Fat</strong></td>
<td>Overall Uniformity</td>
<td>Carcass Weights</td>
<td>Overall Uniformity</td>
<td>Eating Satisfaction</td>
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<td><strong>Overall Palatability</strong></td>
<td>Marbling</td>
<td><strong>Tenderness</strong></td>
<td>Instrument grading</td>
<td>How and where cattle were raised</td>
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<td><strong>Tenderness</strong></td>
<td>Marbling</td>
<td>Market Signals</td>
<td>Weight and Size</td>
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<td><strong>Overall Cutability</strong></td>
<td>External and Seam Fat</td>
<td>Reduced quality due to implants</td>
<td>Segmentation</td>
<td>Lean, Fat and Bone</td>
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<tr>
<td><strong>Marbling</strong></td>
<td>Cut weights</td>
<td>External Fat</td>
<td>Carcass Weights</td>
<td>Cattle Genetics</td>
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</table>
National Beef Quality Audit – 2011

Specific recommendations to reduce barriers and improve beef profitability

- Increase funding to improve *eating satisfaction*
- Use genetics to optimize cutability and *palatability*

**Fit Cattle with Customer Expectations**

- Assist producers with use of selection and management techniques to produce cattle that fit customer expectations and other value-determining attributes
Selection/ Prediction challenges

- Trait measured after slaughter, expensive to measure routinely, low consistency
- Currently USDA grading system (marbling and maturity) is used to predict palatability of beef
  - Changes in consumer preference
  - Limitation in the ability to predict eating quality
  - Limited consumer understanding of the system

**Programs to improve** eating quality
**Ability to better predict** the eating quality level for market purposes

Increase consumer’s confidence that quality expectations are met

Higher quality grade = more tender and palatable meat
Palatability – eating satisfaction

- Warner-Bratzler Shear Force
- Tenderness
- Juiciness
- Connective Tissue
- Beef Flavor
- Off-flavors: painty/fishy, livery/metallic
Sample Collection and Preparation

- Rib sections:
  - Transported to ISU Meat Laboratory, or shipped from California to OSU
  - Fabricated into steaks
  - Frozen at 14d postmortem
Warner-Bratzler Shear Force (WBSF)

- Broiled on impingement oven at 200°C to internal temperature of 68°C
- Cooled at 4°C for 18-24 h
- **Six** 1.27 cm cores removed and sheared
- Average peak load (kg) was analyzed
Sensory Panels

• Cooked similarly to steaks for WBS
• Sessions conducted once or twice per day
• 12 samples served randomly to panelists
• Served to eight member trained panel

Each steak → 8 trained panelists → Average score of all panelists
Sensory Panel Evaluation

• Evaluated juiciness, tenderness, flavor
  • **Juiciness** – 8 point scale (1 = extremely dry and 8 = extremely juicy)
  • **Tenderness** – 8 point scale (1 = extremely tough and 8 = extremely tender)
  • **Connective Tissue** – 8 point scale (1 = abundant and 8 = none)
  • **Beef, Painty/Fishy, and Livery/Metallic Flavors** – 3 point scale (1 = not detectable, 2 = slightly detectable, and 3 = strong)
### Palatability Statistics

<table>
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<tr>
<th>Trait</th>
<th>N</th>
<th>Mean ± SD</th>
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<tbody>
<tr>
<td>WBSF</td>
<td>2,076</td>
<td>3.54 ± 0.77</td>
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<tr>
<td>Tenderness</td>
<td>1,591</td>
<td>5.80 ± 0.59</td>
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<tr>
<td>Juiciness</td>
<td>1,591</td>
<td>5.00 ± 0.49</td>
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<tr>
<td>Connective Tissue</td>
<td>1,591</td>
<td>5.89 ± 0.59</td>
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<tr>
<td>Beef Flavor</td>
<td>1,591</td>
<td>2.50 ± 0.23</td>
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<tr>
<td>Painty</td>
<td>1,591</td>
<td>1.13 ± 0.17</td>
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<tr>
<td>Livery</td>
<td>1,591</td>
<td>1.10 ± 0.12</td>
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Palatability of Beef - goals

1. Genome-wide association (GWAS)
2. Genomic Prediction
3. GWAS signals → underlying biology
Genome-wide association

- Identify **SNPs** and chromosomal regions associated with palatability traits
  - WBSF
  - Tenderness (sensory panel)
  - Juiciness (sensory panel)
  - Connective tissue (sensory panel)
  - Flavor (sensory panel)

- **SVS (SNP & Variation Suite) v8.3.4** (Golden Helix)
  - Mixed Model GWAS using single locus models (**EMMAX**) and multi-locus models (**MLMM**)
# GWAS in SVS - initial steps

## Table of Data

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<tr>
<th>Chromosome</th>
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<th>Strand Versus dbSNP</th>
<th>GenTrain Score</th>
<th>Strand</th>
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<table>
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<tr>
<td>WBSAVG (Quantitation)</td>
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<td>No 2,110 x 53,465</td>
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GWAS in SVS – genotype filtering

![GWAS in SVS - genotype filtering](image-url)
GWAS for WBSF - SVS

- Mixed Model GWAS using a single locus (**EMMAX**) and multi-locus models (**MLMM**)
  - Genomic relationship matrix
  - Contemporary groups
GWAS for WBSF - SVS - output
GWAS for WBSF - SVS

- Mixed Model GWAS using a single locus (EMMAX)
  - Genomic relationship matrix
  - Contemporary groups

Samples scanned: 2,076
Markers scanned: 51,218
Markers analyzed: 51,141
Pseudo-heritability: 0.37
Exploring data - GenomeBrowser
Exploring data - GenomeBrowser
Exploring data - GenomeBrowser
GWAS on other “tenderness” traits

Genetic Markers associated with WBSF

Genetic Markers associated with Tenderness
- CAST
- CAPN3
- SCN3B
- ATL3
- PLA2G16
- CAPN1

Genetic Markers associated with Connective Tissue
GWAS on other palatability traits

Samples scanned: 1,591
Pseudo-heritability: 0.21

Genetic Markers associated with **Juiciness**

Pseudo-heritability: 0.26

Genetic Markers associated with **Flavor**
Palatability of Beef - goals

1. Genome-wide association (GWAS)

2. Genomic Prediction

3. GWAS signals underlying biology
Genomic Prediction - WBSF

Compute Genomic BLUP (GBLUP)

- Compute GBLUP (Genomic Best Linear Unbiased Predictors) of additive genetic merits by sample and of allele substitution effects (ASE) by marker.

- Impute missing genotypic data as:
  - Homozygous major allele
  - Numerically as average value

- Correct for Additional Covariates
  - NUMCONTG

- Correct For Gender
  - Choose Sex Column
  - Select Column
  - Chromosome that is hemizygous for males: X

- Use Pre-Computed Genomic Relationship Matrix
  - GBLUP Genomic Relationship Matrix
  - Select Sheet

- Missing Phenotypes
  - Predict random effects for samples with missing phenotypes
  - Drop samples with missing phenotypes

NOTE: If no pre-computed genomic relationship matrix spreadsheet is selected, a genomic relationship matrix will be computed from the genotype data and used for this analysis.
Samples scanned: 2076
Markers scanned: 51218
Markers analyzed: 51218
(Monomorphic and all-heterozygous markers included above: 77)
Pseudo-heritability: 0.387245
Vg: 0.223871
Ve: 0.35424
Proportion of genetic variance: 0.380596

ASE vs GWAS results

GBLUP ASE

Bayes C-pi ASE
Genomic Prediction

• **Accuracy** of DGV - key to successful application of genomic selection

• Cross validation - assess performance of prediction

• SVS: five-fold cross-validation for evaluation of predictive performance of GBLUP/BayesC for WBSF
5-fold cross-validation - WBSF
Cross-validation - WBSF
Accuracy of gEBV

- Genetic correlation between gEBV and phenotype.
- Bivariate animal model in Wombat

Accuracy: 0.59
Palatability of Beef - goals

1. Genome-wide association (GWAS)

2. Genomic Prediction

3. GWAS signals → underlying biology
Palatability trait

• **8-10 individual traits** (sub-phenotypes):
  - 50,000 SNP effects for each one on >2,000 animals

• **Systems biology**: integration of data sets
  - Holistic view of the system – key players can emerge

**Gene network theory**: use SNP association data to guide the inference of gene regulatory networks

**Association Weight Matrix** *(Fortes 2010)*
multivariate view of GWAS using PCIT (partial correlation and information theory *Reverter & Chan 2008)*
Network Analysis (Palatability)

- All Phenotypes (10 total) + 54k SNPs
  - SVS EMMAX
  - Select top SNPs based on WBSF
    - Matrix of SNP effects (-log10pvalue)
      - 100 SNP by 10 traits
  - PCIT

- Partial Correlation Information Theory (Reverter and Chan, 2008)
- Significant SNPs correlated among multiple sub-phenotypes are important for regulating the overall phenotype
  - Optimized to handle SNPs and genomic windows
Network Analysis (Palatability)

- **Networking and Clustering**
  - Direct correlations visualized in **Cytoscape**
  - Clustering analysis - **MCODE** - highly connected genes
  - Enriched **GO** terms were identified using **DAVID** and visualized using **REViGO**

[Diagram showing the flow of analysis]

1. All Phenotypes (10 total) + 54k SNPs
2. SVS EMMAX
3. Select top SNPs based on WBSF
4. Matrix of SNP effects (-log10pvalue)
   - 100 SNP by 10 traits
5. PCIT
6. Direct and partial correlations among 100 SNPs
7. ENSEMBL annotation of 100 SNPs
8. SNPs in genes
9. SNPs not in genes
10. SNPs ~ 2,500 bp of a gene
11. Cytoscape correlation network
12. DAViD enrichment analysis
13. Gene cluster scoring
14. KEGG Pathway Analysis
15. GO/Biol. Process definitions
Network Visualization

Reference phenotype - Warner-Bratzler Shear Force
Most significant SNPs

• **99** SNPs

Nodes indicate SNP’s
Edges indicate correlations
**SAPS3**: modulates protein phosphatase catalytic subunits

**CAPN1**: modulates proteolysis of cytoskeletal remodeling and signal transduction.

**CHI3L2**: Involved in cartilage biogenesis.

**CA10**: Catalyzes reversible hydration of carbon dioxide in various processes.

**GPHN**: Involved in membrane protein-cytoskeleton interactions.
Conclusions

• Palatability or eating satisfaction - important for the long-term sustainability of beef industry

• Collection of palatability phenotypes on large numbers of animals is still problematic
  • Findings focused on QTL detection rather than genomic prediction

• New methods and approaches to move from SNP signals closer to functional variants
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