“Developing tools to simplify the use of DNA information in beef cattle breeding”

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This work is being funded by Grant 2013-68004-20364 from the USDA National Institute of Food and Agriculture.”
Outline

- Recap: What are we trying to do?
- Let's assume we have success – then what?
- Defining a breeding objective
- Management of recessive conditions in breeding programs
- Utilizing genetic and management tools to optimize mate selection decisions
- Anticipated outcomes of USDA reproduction grant
Genotype-Driven Screens for Embryonic Lethals

- Sequence bulls and identify SNPs that are predicted to have a disruptive effect on protein structure (also called causative DNA sequence variants DSV)
- Develop chip of DSVs and sequence large number of healthy individuals to identify embryonic lethals
- True embryonic lethals should never be observed in the homozygous state among healthy animals and the resulting departure from Hardy-Weinberg equilibrium should be statistically significant
- Carrier status of the sire and dam should have a negative effect on fertility traits
If allele frequency of SNP is 50% A: 50% T then expect 25% AA; 50% AT, 25% TT

If see 33% AA and 66% AT then have a case of missing homozygotes (i.e. TT is likely lethal)
The exact genes and their underlying biological roles in fertilization and embryo development are unknown, but it is assumed that the outcome of inheriting the same haplotype from both parents is failed conception or early embryonic loss.

The reactive approach of attempting to eradicate every animal with an undesirable haplotype is not recommended in light of their economic impact, and is not practical given the likelihood that many more undesirable haplotypes will be found.

Producers should neither avoid using bulls with these haplotypes nor cull cows, heifers, and calves that are carriers, because this will lead to significant economic losses in other important traits.

Computerized mating programs offer a simple, inexpensive solution for avoiding affected matings, so producers should use these programs and follow through on the mating recommendations.
Translational questions that remain assuming success

- All animals carry recessive genetic conditions – how should “embryonic lethals” be managed
- What is the appropriate penalty to put on embryonic lethals when making mating decisions – how to incorporate into mate selection
- What is the frequency of the embryonic lethals in the target population – if small then less important
- Are appropriate decision support tools available for producers???
Where to Go?

Breeding Objective
(Goal of the breeding program)

What traits need to be improved, and how important are different traits relative to each other?
1957 vs. 2001 chickens

1957

2001

43  57  71  85 d.
Annual Production Derived from 1 Pedigree
Broiler Female

1 Female
23.4 Females
725 Females
24,809 Females
3,064,000 Broilers

Great Grand Parents
Grand Parents
Parents
Broilers

3 years

Image kindly provided by Dr. Rachel Hawkin, Cobb-Vantress

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Animal Genomics and Biotechnology Education
Genetic selection to improve bird’s health and welfare

Throughout the 1990’s and in the new millennium Cobb added additional selection traits related to chicken wellbeing including

- more sophisticated measurements of leg health (e.g. tibial dyschondroplasia (TD) and femoral head necrosis (FHN)),
- walking ability,
- cardiovascular fitness (e.g. ascites),
- skin condition (e.g. foot pad dermatitis), and
- disease resistance.

These trait measurements are now routine parts of Cobb broiler breeding selection programs and include X-ray testing for TD, FHN evaluation, gait assessment, blood oxygen measurement for ascites control and foot pad dermatitis grading. Associated with this, Cobb incidence of TD in pedigree lines decreased, field reports of ascites declined and company regional managers reported reductions in leg problems in each world region.

http://www.cobb-vantress.com/a-better-world/overview/blog/detail/a-better-world/2012/12/28/genetic-selection-to-improve-bird-s-health-and-welfare
Breeding Objective

“A breeding objective need not be economic. For example, in many companion animal species it is tempting to believe that the breeding objective must be the maintenance of a ridiculous appearance and congenital abnormalities!”

(John Gibson, UNE)
Given this information......
Which do you think is my dog?

1. 
2. 
3. 
4. 

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Animal Genomics and Biotechnology Education
What emphasis do you think milk yield given in the national US dairy selection index?

1. 0
2. 25%
3. 50%
4. 66%
5. 75%
6. 100%
Year that genetic rankings began and emphasis placed on dairy traits in 2010 US national dairy selection indexes

<table>
<thead>
<tr>
<th>Trait</th>
<th>Year begun</th>
<th>Emphasis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Milk yield</td>
<td>1935</td>
<td>0</td>
</tr>
<tr>
<td>2. Milk Fat</td>
<td>1935</td>
<td>16</td>
</tr>
<tr>
<td>3. Milk Protein</td>
<td>1977</td>
<td>19</td>
</tr>
<tr>
<td>5. Udder Shape and Support</td>
<td>1983</td>
<td>7</td>
</tr>
<tr>
<td>6. Feet and Leg Conformation</td>
<td>1983</td>
<td>4</td>
</tr>
<tr>
<td>7. Body Size/Weight</td>
<td>1983</td>
<td>-6</td>
</tr>
<tr>
<td>8. Productive Life/Longevity</td>
<td>1994</td>
<td>22</td>
</tr>
<tr>
<td>10. Daughter Pregnancy Rate/Fertility</td>
<td>2003</td>
<td>11</td>
</tr>
</tbody>
</table>


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Animal Genomics and Biotechnology Education
Historically not all beef cattle breeding objectives have been economic.

Photo taken in 1949 at Red Bluff Bull Sale, CA. Kindly provided by Cathy Maas from Crowe Hereford Ranch, Millville, CA.


1986. "Coblepond New Yorker" weighed 2529 lbs and measured 65 inches tall at 35 mos. (Frame 10) when he was Denver Champion.

1988 Grand Champion Bull, National Polled Hereford Show (frame 10).

Images from Harlan Ritchie’s historical review of type https://www.msu.edu/~ritchieh/historical/cattletype.html
We can make genetic changes in our cattle (and our dogs) - the question is are we making profitable change?

Wilt Chamberlain

Willie Shoemaker

Killed same day at IBP in Iowa:
The small female weighed 835 lbs and was extremely fat. The large male weighed 1900 lbs and was very lean.

Images from Harlan Ritchie’s historical review of type
https://www.msu.edu/~ritchieh/historical/cattletype.html
Which trait group should be given the most selection emphasis in US beef cattle production systems?

1. Appearance
2. Growth
3. Reproduction
4. Carcass traits
5. Depends
6. None of above
Need to make sure you are using the right index for your production system

- It is important to understand that in most cases indexes only encompass postweaning and carcass performance. Other factors drive profitability, especially fertility.
- Cow-calf producers keeping replacement heifers and selling calves at weaning should have a relative economic emphasis of **47% on reproduction**, 24% on growth, and 30% on carcass traits.
- Producers in an integrated system should have a relative economic emphasis of **31% on reproduction**, 29% on production, and 40% on carcass traits (Melton, 1995).
- This relative emphasis will depend on how much the value derived from genetic gain in feedlot and carcass traits is shared with the producer in the integrated system.

Where to Go?

How to Get There?

Trait Measurement
- Which traits
- Which animals
- Males versus females
- Progeny test

Estimation of EPDS (breeding value)
- Phenotypes
- Pedigree
- BLUP
- DNA information

Reproductive Technologies
- Artificial insemination
- Multiple Ovulation/Embryo Transfer

Selection/Culling/Mate Selection
Based on genetic evaluation
- Balancing the rate of genetic change ($\Delta G$) and inbreeding

Image adapted from “Genetic Evaluation and Breeding Program Design” (2011).
University of New England, Australia
Modified from slide provided by Dr. Brian Kinghorn, UNE, Australia
INDEXES. Multi-trait selection indexes combine EPDs for several traits into a single economic value. The index values are interpreted like EPDs; the difference in index value between two bulls is the expected difference in average dollar value of their progeny, when the bulls are bred to similar cows. Indexes are expressed in dollars per head, and higher indexes mean a higher dollar value per head. An index value only has meaning when it is compared to the index value of another animal of the same breed. In the US indexes are currently calculated for Angus, Charolais, Gelbvieh, Hereford, Limousin, Simmental, and SimAngus bulls.
Example: $Indexes calculated for Australian Angus animals.

- **Long Fed / CAAB Index ($)** - Estimates the genetic differences between animals in net profitability per cow joined for an example high fertility self-replacing commercial Angus herd in temperate Australia targeting pasture grown steers with a 270 day feedlot finishing period for the high quality, high marbled Japanese export market. Significant emphasis is placed on marbling and 600 day growth.

- **Terminal Index ($)** - Estimates the genetic differences between animals in net profitability for an example commercial crossbred herd where no animals are kept for breeding. For example using Angus bulls over tropical cows targeting pasture grown steers and heifers with a 100 day feedlot finishing period. Progeny are assumed marketed at 1320 lb live weight at 23 months of age. Emphasis is on growth and carcass yield with no weighting placed on calving ease, female fertility or milk.
Long Fed / CAAB Index ($) - Estimates the genetic differences between animals in net profitability per cow joined for an example high fertility self-replacing commercial Angus herd in temperate Australia targeting pasture grown steers with a 270 day feedlot finishing period for the high quality, high marbled Japanese export market. Significant emphasis is placed on marbling and 600 day growth.

<table>
<thead>
<tr>
<th>Table 2 - Long Fed / CAAB Index - Profit Drivers</th>
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</thead>
<tbody>
<tr>
<td><strong>Sale Liveweight Dir.</strong></td>
</tr>
<tr>
<td><strong>Sale Liveweight Mat.</strong></td>
</tr>
<tr>
<td><strong>Dressing %</strong></td>
</tr>
<tr>
<td><strong>Saleable Meat %</strong></td>
</tr>
<tr>
<td><strong>Fat Depth (Rump)</strong></td>
</tr>
<tr>
<td><strong>Cow Weaning Rate</strong></td>
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<tr>
<td><strong>Marbling Score</strong></td>
</tr>
<tr>
<td><strong>Cow Survival Rate</strong></td>
</tr>
<tr>
<td><strong>Cow Weight</strong></td>
</tr>
<tr>
<td><strong>Calving Ease Dir.</strong></td>
</tr>
<tr>
<td><strong>Calving Ease Mat.</strong></td>
</tr>
</tbody>
</table>
The range of traits for which BREEDPLAN (Australia) produces EPDs is shown below.

BREEDPLAN® TRAITS

**GROWTH**
- Birth weight
- Growth - Weaning
- Growth - Yearling
- Growth - Final
- Maternal (Milk)
- Mature cow weight

**FERTILITY**
- Scrotal size
- Days to calving
- Gestation length
- Calving ease - direct
- Calving ease - daughter

**CARCASS**
- Carcass weight
- Fat depth - Rump
- Fat depth - Rib
- Retail beef yield
- Intramuscular fat

**OTHER**
- Net feed intake
- Docility
- Flight time
- Shear force
- Conformation
Long Fed / CAAB Index ($) - Estimates the genetic differences between animals in net profitability per cow joined for an example high fertility self-replacing commercial Angus herd in temperate Australia targeting pasture grown steers with a 270 day feedlot finishing period for the high quality, high marbled Japanese export market. Significant emphasis is placed on marbling and 600 day growth.

Table 3 - Long Fed / CAAB Index - EBV Weightings

<table>
<thead>
<tr>
<th>Trait</th>
<th>EBV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calving Ease Dir.</td>
<td>14%</td>
</tr>
<tr>
<td>Calving Ease Mat.</td>
<td>9%</td>
</tr>
<tr>
<td>Birth Weight</td>
<td>1%</td>
</tr>
<tr>
<td>Milk</td>
<td>1%</td>
</tr>
<tr>
<td>200 Day Growth</td>
<td>-2%</td>
</tr>
<tr>
<td>400 Day Weight</td>
<td>-2%</td>
</tr>
<tr>
<td>600 Day Weight</td>
<td>26%</td>
</tr>
<tr>
<td>Intramuscular Fat</td>
<td>19%</td>
</tr>
<tr>
<td>Days to Calving</td>
<td>-1%</td>
</tr>
<tr>
<td>Scrotal Size</td>
<td>1%</td>
</tr>
<tr>
<td>P8 Fat Depth</td>
<td>1%</td>
</tr>
<tr>
<td>Eye Muscle Area</td>
<td>7%</td>
</tr>
<tr>
<td>Retail Beef Yield</td>
<td>5%</td>
</tr>
<tr>
<td>Mature Cow Weight</td>
<td>-12%</td>
</tr>
</tbody>
</table>
Terminal Index ($) - Estimates the genetic differences between animals in net profitability for an example commercial crossbred herd where **no animals are kept for breeding**. e.g. Angus bulls over tropical cows targeting pasture grown steers and heifers with a 100 day feedlot finishing period. Progeny are assumed marketed at 1320 lb live weight at 23 months of age. Emphasis is on growth and carcass yield **with no weighting placed on calving ease, female fertility or milk.**

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**Terminal Index - Profit Drivers**

- **Sale Liveweight Dir.**: 36%
- **Sale Liveweight Mat.**: 0%
- **Dressing %**: 25%
- **Saleable Meat %**: 28%
- **Fat Depth (Rump)**: 7%
- **Cow Weaning Rate**: 0%
- **Marbling Score**: 2%
- **Cow Survival Rate**: 0%
- **Cow Weight**: 0%
- **Calving Ease Dir.**: 2%
- **Calving Ease Mat.**: 0%
Maternal CAAB index versus Terminal Index

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Develop selection indexes that incorporate age-dependent fertility of females (including heifers) in the context of cow-calf and feedlot production with quality and yield grade based marketing of carcass beef. Thus, users of the research results will be provided economically rational emphases for fertility, growth, efficiency, and carcass traits upon which to base selection and mating decisions.

"To date, the consideration of reproduction has been cursory in selection indexes used by providers of genetic evaluation to beef breeders and producers. Thus, it is proposed that breeding objectives that explicitly model age-specific reproductive rate as a basis for selection indexes be developed. The indexes will consider the complete life-cycle in a production system that extends from conception to product. Because reproduction is measured late in life relative to the time that selection decisions are made, consideration of phenotypic and molecular indicator traits will be of considerable importance."

Dr. Mike MacNeil

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When making selection decisions to improve fertility – which group of cattle should selection focus upon?

1. Bulls
2. Cows
3. Heifers
Genetic composition of the herd: 
87% of genetic composition of calf crop is determined by the sires used over the last 3 generations.

Maternal great-grand sires (12.5%)
Maternal grand sires (25%)
Sires (50%)

Image adapted from "More Beef from Breeding" workshop (2007). Meat and Livestock (MLA), Australia
Time line for beef breeding

Bull purchase/selection

Progeny born

Progeny slaughtered

Female progeny used for breeding

Classic “decision” systems

- **Targets**
- **Data**
  - Breeding Objective
- **Parameters**
  - Sets of Rules
    - Sire use
    - Dams per sire
    - Avoid inbreeding
    - Trait distributions
    - Avoid genetic defects
    - Reproductive technologies
    - …etc.

ACTION

Not always arriving at optimal solution to complex problem

Modified from slide kindly provided by Dr. Brian Kinghorn, UNE, Australia
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Genetic implications of recessive genetic factors

“Carrier animals….their overall breeding value worth may outweigh the economic value of carrier status”


Need to penalize carrier animals appropriately (not prohibit their use entirely) and let mate selection software optimize their use in the breeding programs.
American Angus Defect Codes

- Arthrogryposis Multiplex carrier = AMC
- Arthrogryposis Multiplex free = AMF
- Arthrogryposis Multiplex affected = AMA

Photo used with permission from http://www.angus.org.
It will be essential to develop methods that prioritize SNP variants based on the likelihood that they contribute to disease

- The frequencies of different classes of variations in ten case and ten control human genomes were compared (K. V. Shianna et al., unpublished data).
- There were 383,913 variants (single nucleotide variants and indels) present in at least two cases and no controls.
- However, if testing is restricted to only variants that affect the coding sequence (i.e. missense mutations), this number drops to 2,354.
- If testing is restricted to only protein-truncating variants (i.e. nonsense mutations), the number drops further to 152.

Early extension education about dwarfism explaining carriers and inheritance

Image from Special Collections University Libraries, Virginia Tech: http://spec.lib.vt.edu/imagebase/agextension/boxseven/screen/AGR3618.jpg
If you breed a curly calf carrier cow (AMC) to an curly calf free bull (AMF), what is the chance that the offspring will be stillborn as a result of being curly calf?

1. 0
2. ¼ (25%)
3. ½ (50%)
4. 2/3 (66%)
5. ¾ (75%)
6. 1 (100%)
If you breed a curly calf carrier cow (AMC) to an curly calf free bull (AMF), what is the chance that the offspring will be stillborn as a result of being curly calf?

1. 0
2. $\frac{1}{4}$ (25%)
3. $\frac{1}{2}$ (50%)
4. $\frac{2}{3}$ (66%)
5. $\frac{3}{4}$ (75%)
6. 1 (100%)

Results from a typical producer meeting
Average Long Fed CAAB Index for sires by birth year

Angus Australia provides probability data on animal in database

Figure courtesy of Carel Teseling, Angus Australia

GeneProb used to estimate the probability of every animal in the database being a carrier based on all ancestor and descendant DNA test results

MateSel integrates complex breeding issues into a single, easy to use, decision making framework. Technical, logistical and economic issues compete for attention in a system that can be guided by the breeder, with the resulting mating list covering decisions on items like semen purchase, bulls used, animal selection/culling, forming mating groups and mate allocation, genetic gain (Indexes), genetic diversity, inbreeding, trait distributions, genetic defect management, logistical constraints and costs. The resulting mating lists optimize the matings for the candidate animals while allowing for all of these variables and constraints.
Mate Sel: Balancing inbreeding and genetic merit – the frontier gives the unconstrained solution.

- Select only the very best AI bull for all breedings.
- Select a number of bulls from many different families.

Looking at index and pedigree only.
Finding the right balance

- The formal breeding objective
- Inbreeding
- Additional constraints e.g. use no animal with a genetic defect in pedigree

Mate selection tool shows you the ‘opportunity cost’ of imposing non-optimal constraints on mate selection
Develop software to handle the mutations discovered in this project, and deliver selection and mating recommendations to US beef breeders that exploit this information optimally in competition with other factors of importance (e.g. trait merit, genetic diversity (inbreeding), genetic defects and recessive lethals, logistical constraints, semen costs, etc.)

"Adapt mate selection methodology and develop software that can be used to optimize the rate of genetic gain (using the selection indexes developed by Mike MacNeil) and mate allocation with a key objective being to reduce both the phenotypic expression and allele frequency of the lethals identified in the project. Equally important, this will be carried out in concert with other important issues such as the management of trait merit, genetic diversity, other genetic defects, genome-wide inbreeding, logistical constraints and costs."

Dr. Brian Kinghorn
Multiple-trait selection indexes that include all of the economically-relevant traits that influence the profitability of US beef cattle production are integral to this optimization and will be available via the DGVs (DNA sequence variants) produced for the 10,000 participating cows.

Indexes provide an economic evaluation of the genetic differences among sires, and an objective method for determining likely differences in the profitability of progeny from different sires conditional on the breeding objective.

It is envisioned that the development of the decision support software proposed in this project based on genotype information and economic indexes will provide a platform to facilitate the incorporation of the results of other genomic projects into national cattle evaluation.

Decision support software is essential to encourage more widespread industry adoption of DNA technologies by providing a unified approach to appropriately weight the relative economic value of traits in the breeding objective against potential deleterious recessives, and suggest an optimal breeding scheme based on all available information.

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QUESTIONS

This project is funded by National Research Initiative Grant # 2013-68004-20364 from the USDA National Institute of Food and Agriculture.”
More than 98% of the genome does not encode protein sequences, including most intergenic DNA and sequences within introns.
The DNA sequence of a gene can be altered in a number of ways. Gene mutations have varying effects, depending on where they occur and whether they alter the function of essential proteins.
Missense mutation (as compared to synonymous)  
This type of mutation is a change in one DNA base pair that results in the substitution of one amino acid for another in the protein made by a gene.
Nonsense mutation

A nonsense mutation is also a change in one DNA base pair. Instead of substituting one amino acid for another, however, the altered DNA sequence prematurely signals the cell to stop building a protein. This type of mutation results in a shortened protein that may function improperly or not at all.
Insertion

An insertion changes the number of DNA bases in a gene by adding a piece of DNA. As a result, the protein made by the gene may not function properly.
**Deletion**

A deletion changes the number of DNA bases by removing a piece of DNA. Small deletions may remove one or a few base pairs within a gene, while larger deletions can remove an entire gene or several neighboring genes. The deleted DNA may alter the function of the resulting protein(s).

![Deletion mutation diagram](image_url)
Duplication

A duplication consists of a piece of DNA that is abnormally copied one or more times. This type of mutation may alter the function of the resulting protein.
Frameshift mutation

This type of mutation occurs when the addition or loss of DNA bases changes a gene’s reading frame. A reading frame consists of groups of 3 bases that each code for one amino acid (i.e. codon). A frameshift mutation shifts the grouping of these bases and changes the code for amino acids. The resulting protein is usually nonfunctional. Insertions, deletions, and duplications can all be frameshift mutations.
Microsatellites (SSR)

Nucleotide repeats are short DNA sequences that are repeated a number of times in a row. For example, a trinucleotide repeat is made up of 3-base-pair sequences, and a tetranucleotide repeat is made up of 4-base-pair sequences. This type of mutation can cause the resulting protein to function improperly.
“Commercial Heifer Selection Using Genomics"

- What traits are of importance for heifer replacement selection?
- What selection criteria are currently available?
- What traits are genomic predictions available for?
- What is the value of genetic improvement in commercial females?
What traits are of importance for heifer replacement selection?

- Has to be big enough to breed as a yearling
- Has to be phenotypically/structurally sound

**THEN IF THERE ARE ANY LEFT – CAN CONSIDER SELECTION**

- Reproductive traits are sex-limited, lowly heritable, and some are expressed quite late in life.
  - age at first calving
  - reproductive success
  - replacement rate

i.e. type of traits benefit most from genomics!
Improving the accuracy of genomic selection?

According to a simulation presented by Meuwissen and Goddard, a 40% gain in accuracy in predicting genetic values could be achieved by using sequencing data instead of data from 30K SNP arrays alone.

Furthermore, by using whole-genome sequencing data, the prediction of genetic value was able to remain accurate even when the training and evaluation data were 10 generations apart: observed accuracies were similar to those in which the test and training data came from the same generation.

According to the authors, "these results suggest that with a combination of genome sequence data, large sample sizes, and a statistical method that detects the polymorphisms that are informative..., high accuracy in genomic prediction is attainable"