Update on USDA Fertility Project

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2015 NBCEC Brown Bagger

Identification and management of alleles impairing heifer fertility while optimizing genetic gain in Angus cattle

USDA-NIFA Award #2013-68004-20364

JF Taylor, DS Brown, MF Smith, RD Schnabel,
SE Poock, JE Decker, FD Dailey, and DJ Patterson
University of Missouri

AL Van Eenennaam
University of California, Davis

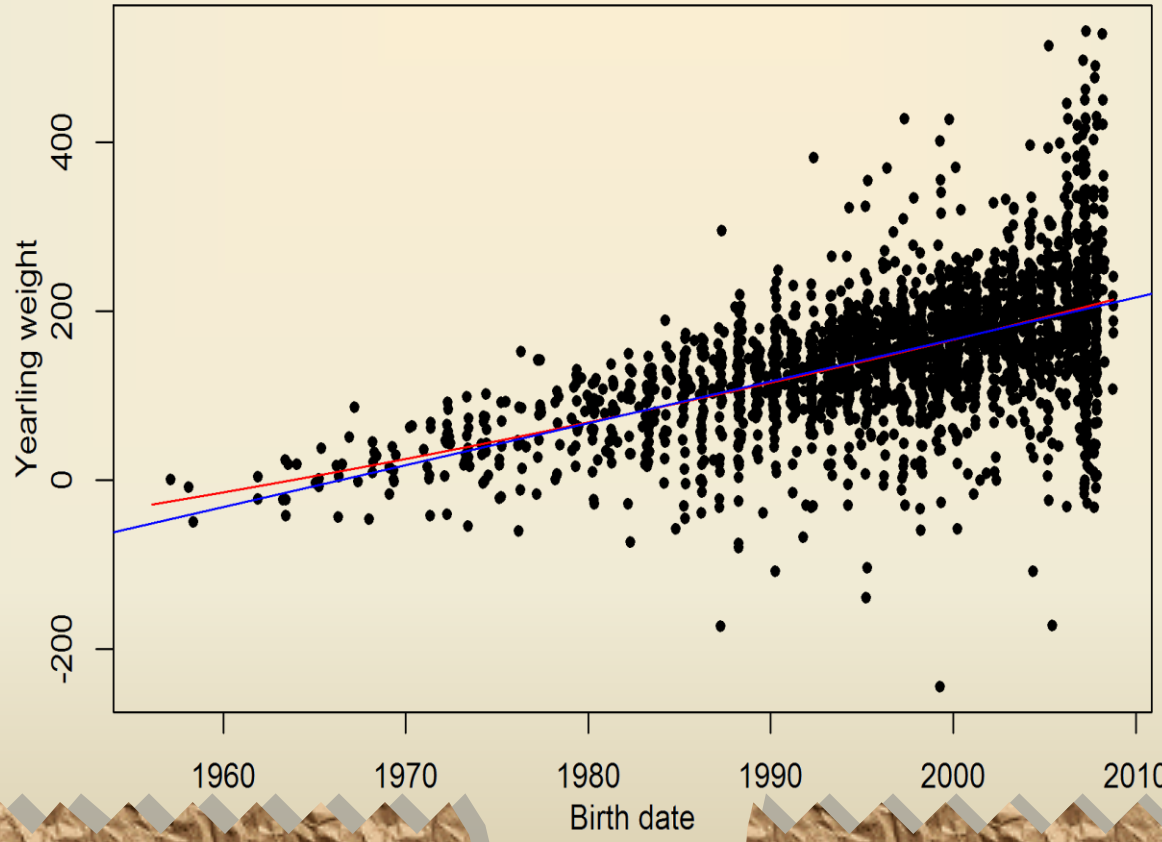
MM Rolf
Oklahoma State University

BP Kinghorn
University of New England, NSW, Australia

MD MacNeil
Miles City, MT

Deregressed EBVs for Yearling Weight of 2,755 registered Angus bulls demonstrates that breeders have achieved an average increase of 4.96 lb per year (blue line) over a 50 year period.

Dramatic Genetic Change

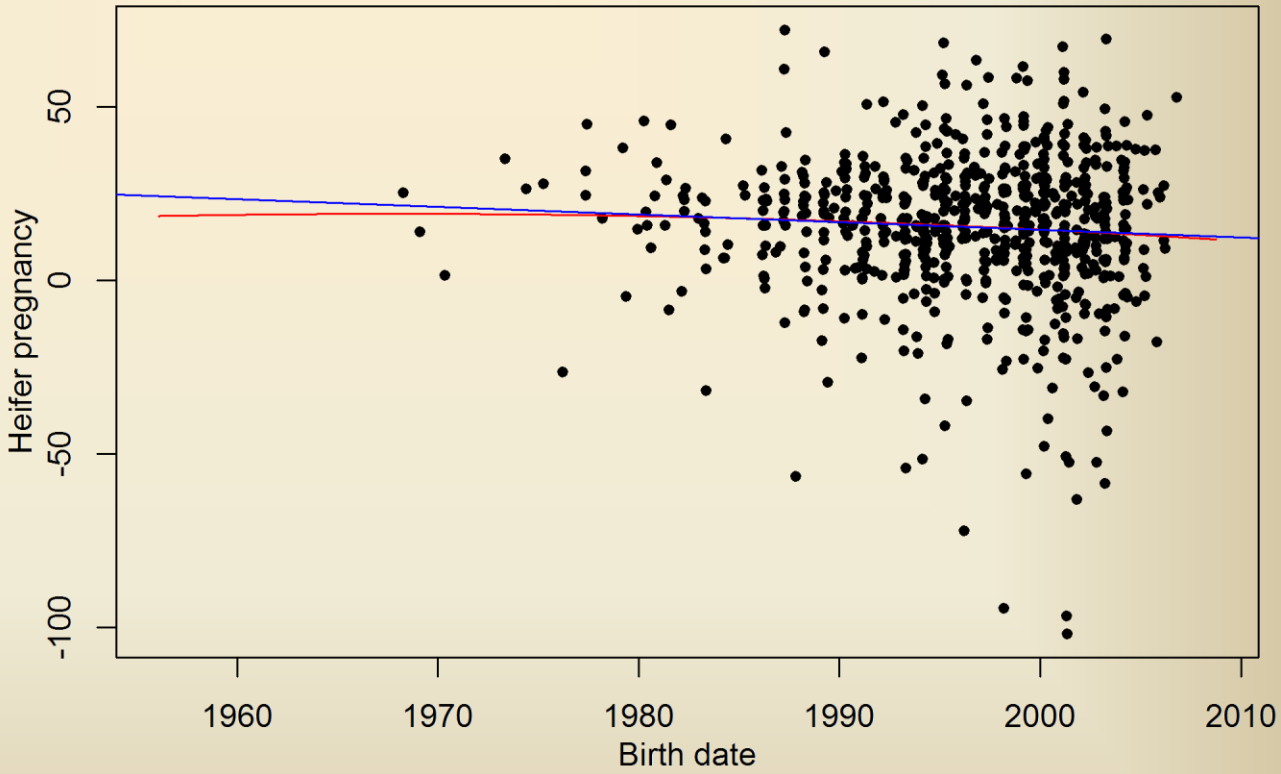


Deregressed EBVs for Heifer Pregnancy Rate for 698 registered Angus bulls indicates that Angus female fertility has decreased by 0.22% per year for about the last 25 years.

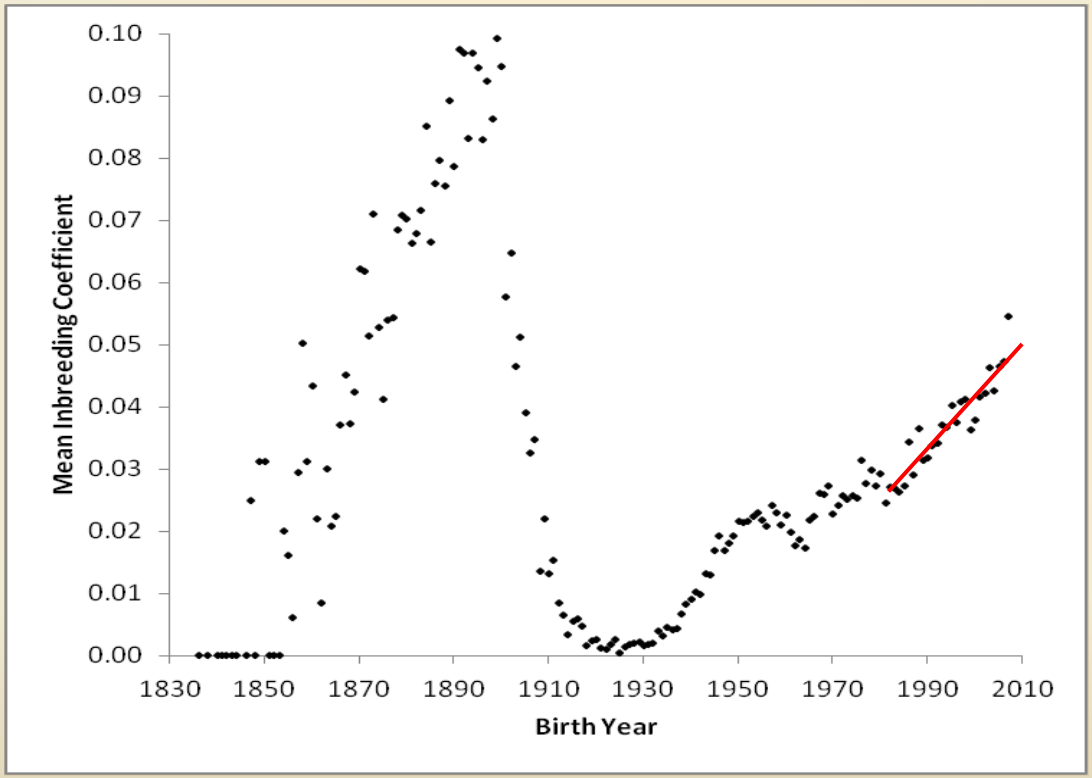
<u>Possible Cause(s)</u>

Correlated response to selection?

Accumulation of inbreeding?



Mean inbreeding coefficients by birth year for 76,083 Angus animals forming a 64 generation pedigree including 3,570 genotyped animals. Effective population size (N_e = 116.15 ± 0.04) was estimated for animals born ≥1980.



Consequences of Inbreeding

- Increases probability of alleles being homozygous
  - As with all lowly heritable traits (like fertility), reduces fitness

Table 1. Summary of heritability and level of heterosis by trait type.[a]

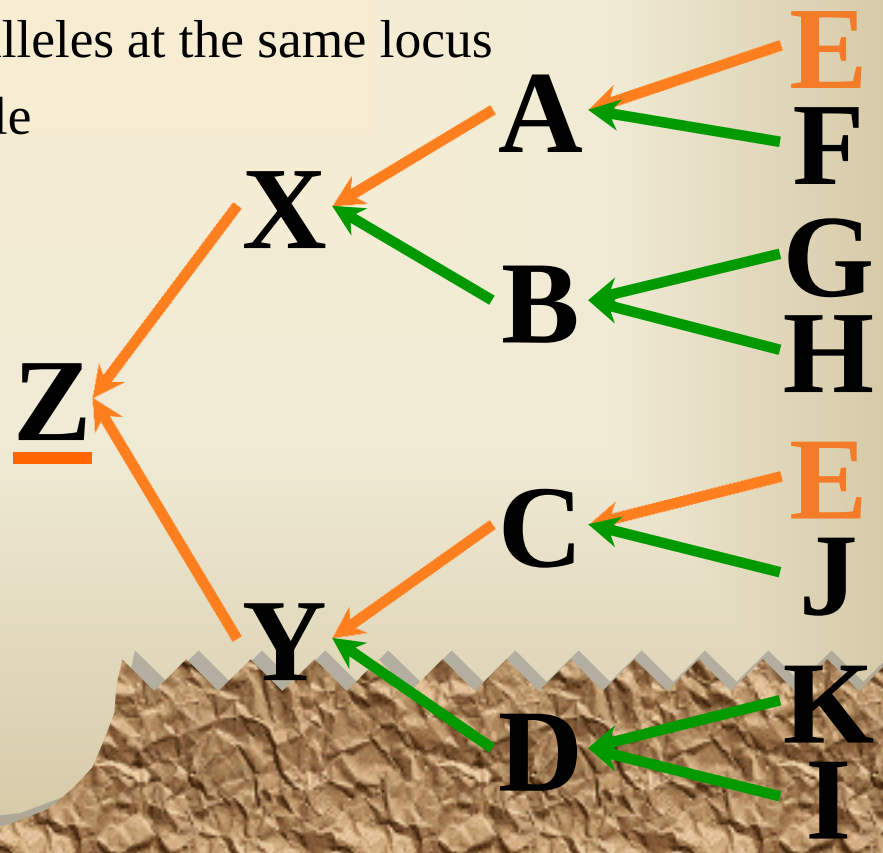
Trait	Heritability	Level of Heterosis	Inbreeding Depression
Carcass/end product	High	Low (0 to 5%)	Low
Skeletal measurements			
Mature weight			
Growth rate	Medium	Medium (5 to 10%)	Medium
Birth weight			
Weaning weight			
Yearling weight			
Milk production			
Maternal ability	Low	High (10 to 30%)	High
Reproduction			
Health			
Cow longevity			
Overall cow productivity			

[a] Adapted from Kress and MacNeil, 1999.

Source: NBCEC Sire Selection Manual

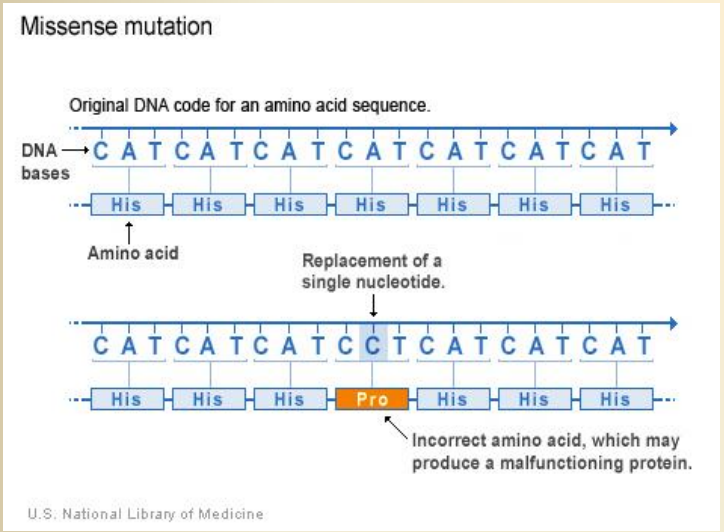
Effects of Inbreeding Accumulation

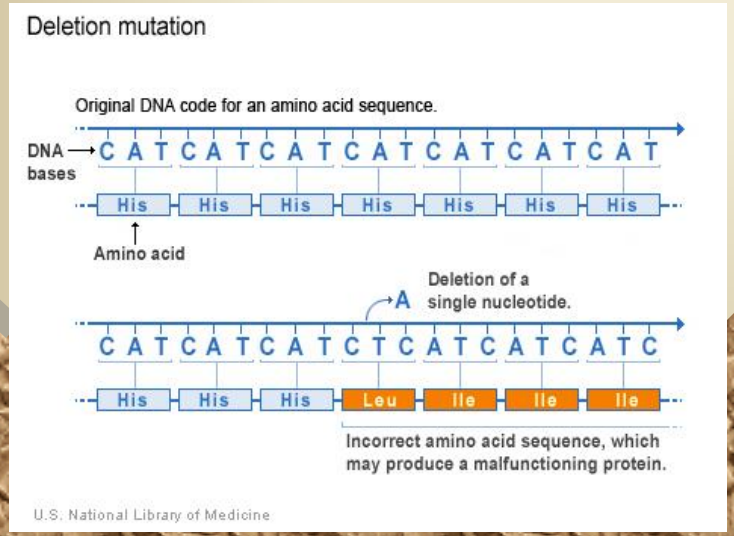
- Increases probability of alleles being homozygous
  - As with all lowly heritable traits, reduces fitness
  - Increases odds of alleles being identical by descent
    - Increases odds of two broken alleles at the same locus
    - Does not cause the broken allele

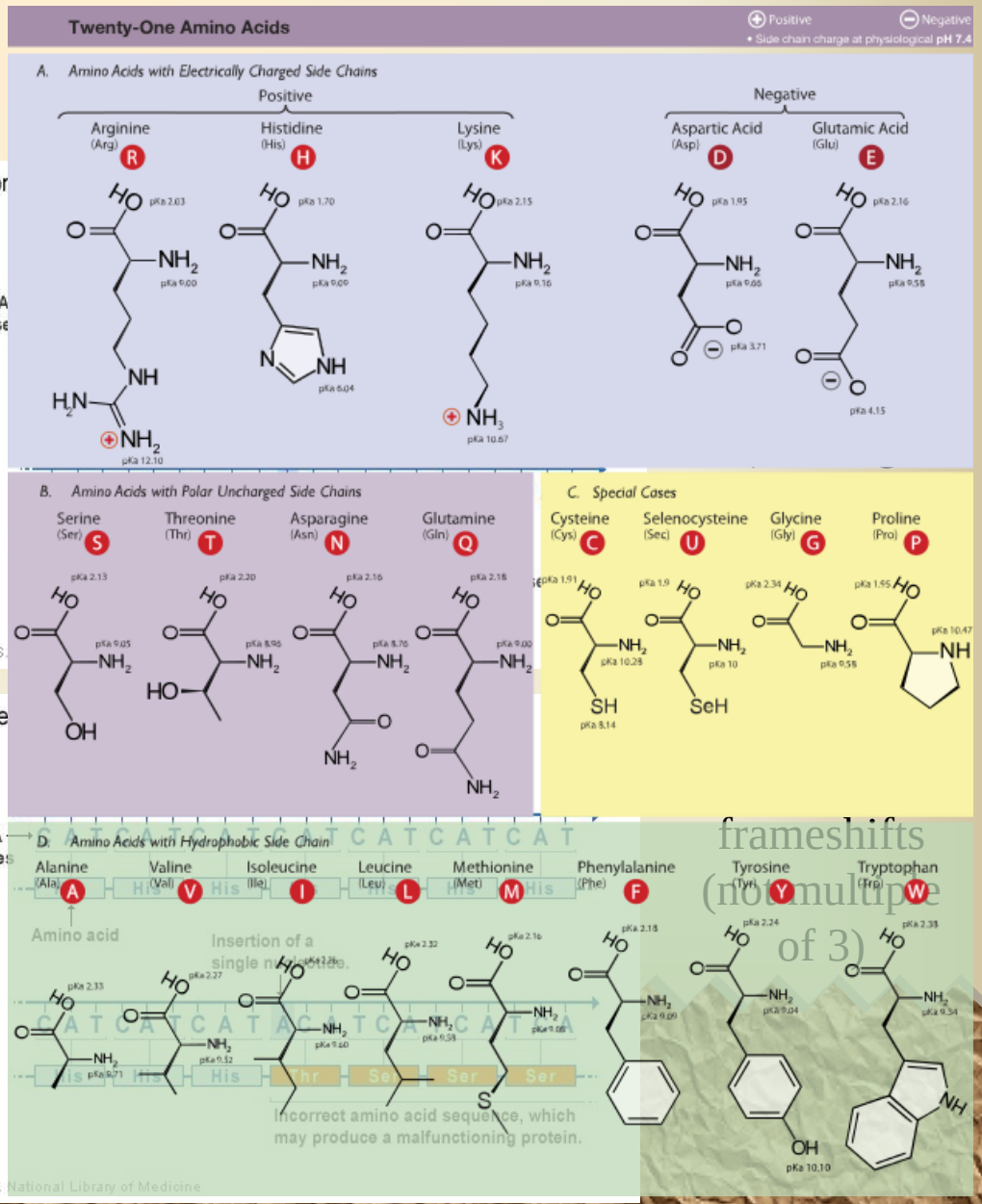


Broken Genes

- How do you break a gene?



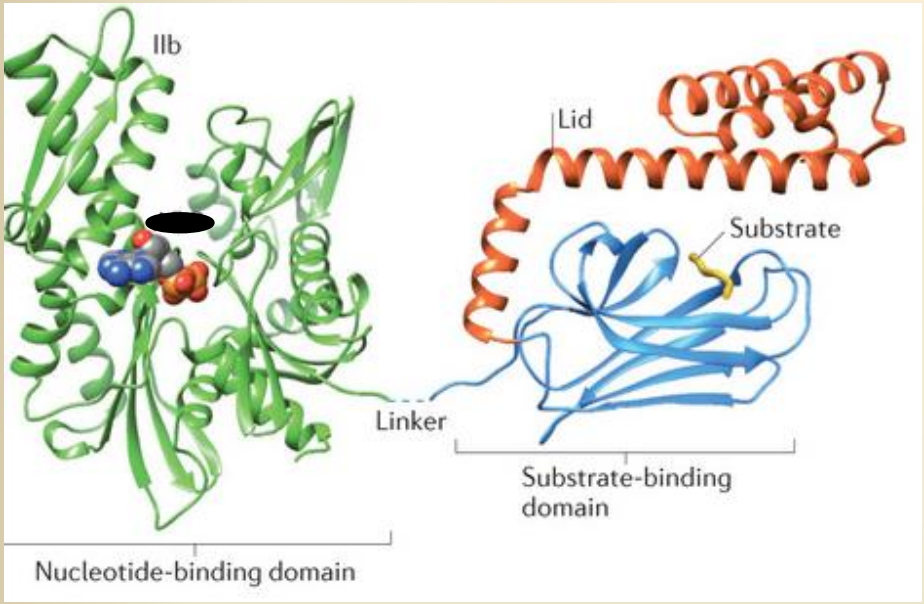




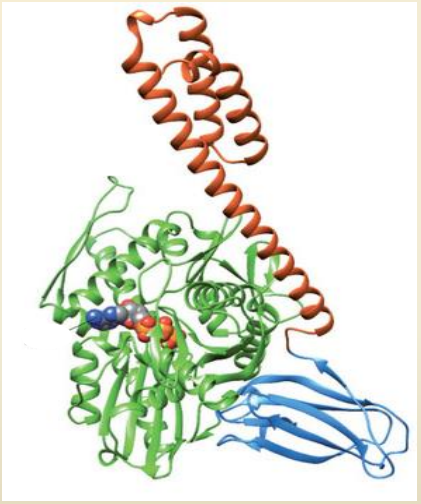
frameshifts (not multiple of 3)

Results from Broken Genes

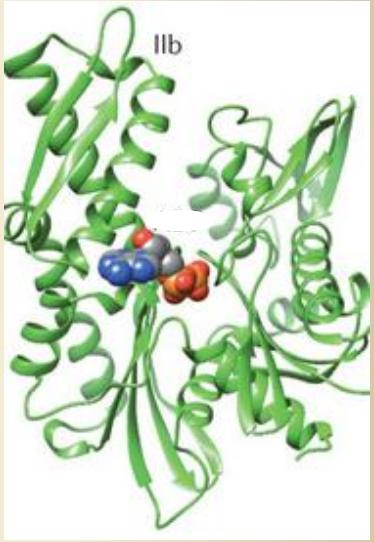
- Truncations, improper folding



Improper Folding



Truncated



Consequences of Inbreeding

- Called Loss of Function Mutations (LOF)
- Can be one of two forms

- Not Critical for Life
  - Will see all genotypes in the population (AA, AB, and BB)
  - Animals may have reduced performance or other deleterious effects, but are functional organisms

- Critical for Life
  - Animals cannot survive without at least one fully functional version of these genes
  - Presumably calves that are homozygous for a loss of function (LOF) allele will abort, be born dead, die soon after birth, or will never be observed in live animals

The Case of the Missing Homozygotes

$p = frequency\ of\ normal\ allele$ (0.95)
$q = frequency\ of\ broken\ allele$ (0.05)

$p^2 = frequency\ of$ homozygous normal
$2pq = frequency\ of\ heterozygotes$
$q^2 = frequency\ of$ homozygous broken

Genotype 10,000 animals-
Expect to see:
9,025 homozygous
950 heterozygotes
25 alternate homozygote

Genotype 10,000 animals for lethal-
You would see:
9048 homozygous normal
952 heterozygotes
0 homozygous broken

Need lots of animals to test this!

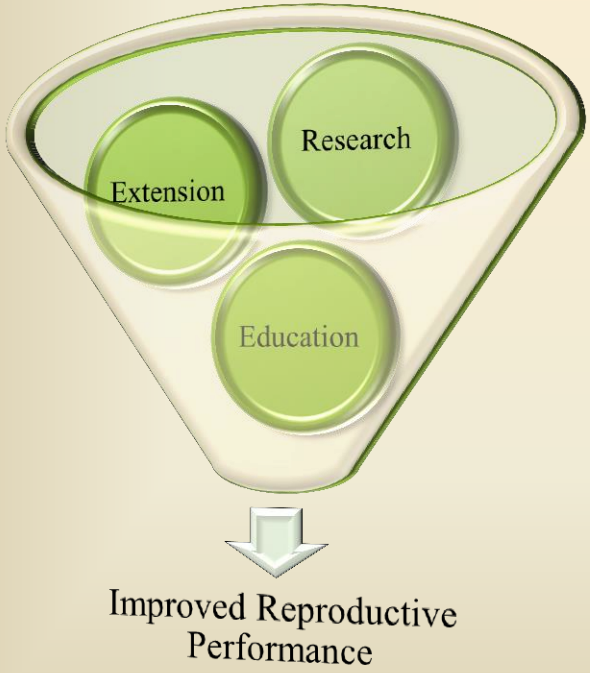
Project Goals and Background

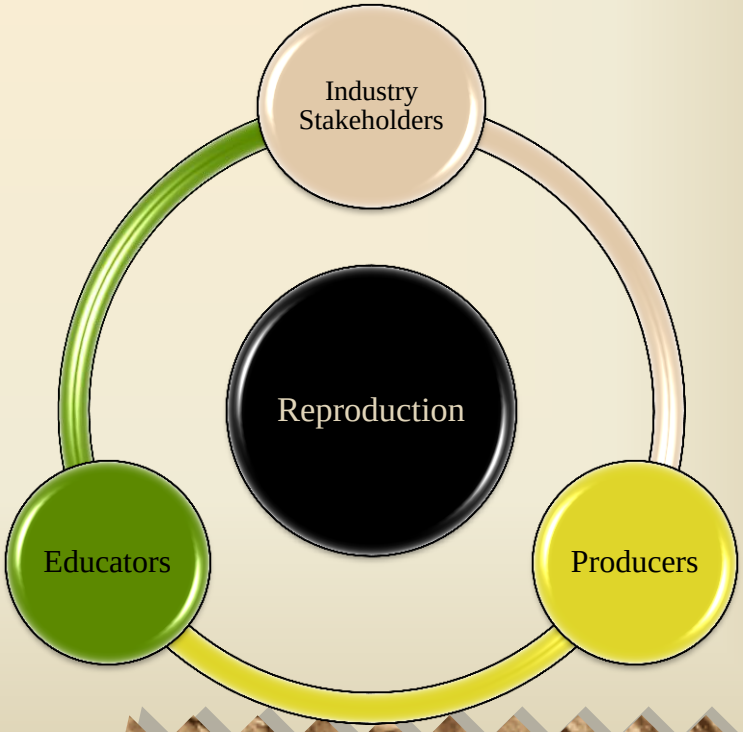
- Improve reproductive rate of US beef cattle by identifying and managing broken alleles (LOF mutations)
  - Does not sacrifice performance in other ERT
  - Improves overall profitability of the cowherd
- No secret that reproduction is a very important trait in the cowherd

- Managing LOF mutations can assist in
  - Maximizing the number of females that conceive early in the breeding season
  - Maintenance of pregnancies that are achieved

How to Get There?

- Integrated approach
  - Research, Extension and Education components
  - Partnerships with educators, industry stakeholders and producers





Specific Aims (Research)

1. • Sequence highly-influential bulls in Angus and other breeds
2. • Identify candidate LOF alleles never observed in homozygous form
3. • Validate candidate LOF alleles through genotyping large population of phenotyped heifers, remove those found as homozygous
4. • Develop EPD and index selection tools for fertility
   • Mate selection software (MateSel)

Progress to Date

- Whole Genome Sequencing (bulls)
  - 109 Angus bulls have been sequenced
  - Several other breeds sequenced through cooperative agreements
  - Genomes contributed from collaborators at Genome Canada, USDA/BARC and 18 breed associations

- *In total 267 bulls representing 18 breeds have been sequenced*

Sequencing Status

Breed	N	Number of reads	Total bases	Total coverage	Average coverage
Angus	109	77,930,820,090	7,694,958,893,355	2,653.43	26.80
Red Angus	14	4,430,950,144	441,846,880,499	152.36	10.88
Hereford	18	14,775,544,682	1,390,024,023,122	479.32	26.63
Limousin	12	3,704,169,818	357,264,463,240	123.19	10.27
Charolais	11	8,061,833,430	802,164,255,493	276.61	25.15
Simmental	11	8,902,705,282	885,698,817,042	305.41	27.76
Gelbvieh	8	6,366,906,096	633,479,558,830	218.44	27.31
Maine Anjou	5	4,061,220,172	403,867,224,031	139.26	27.85
Romagnola	4	901,554,762	89,666,842,589	30.92	7.73
Shorthorn	2	1,370,128,728	136,274,291,678	46.99	23.50
Beefmaster	10	8,351,392,646	830,865,082,100	286.51	28.65
Holstein	25	3,224,948,436	320,796,806,908	110.62	4.42
Jersey	9	1,399,450,902	139,150,036,295	47.98	5.33
Ndama	1	739,233,320	73,483,493,461	25.34	25.34
Brahman	11	1,871,667,422	167,772,161,118	57.85	5.26
Nelore	8	1,668,006,036	165,728,918,125	57.15	7.14
Gir	6	1,583,737,248	157,449,065,756	54.29	9.05
Bison	3	3,242,100,744	322,544,004,793	111.22	37.07
Total	267	152,586,369,958	15,013,034,818,435		

Progress to Date

- Identify candidate LOF alleles
  - Identify and evaluate mutations not found in homozygous form
  - Identify those with predicted disruptive effects on protein structure (frameshifts, deletions, insertions, premature stop codons, non-synonymous mutations)

- Developed SNP chip with predicted LOF DNA sequence variants (DSV) for validation of LOF mutations
  - Remove any that are found in homozygous state in larger population of healthy animals
  - Significant deviations from HWE should also be a clue (missing homozygotes)

Chip Data Sources

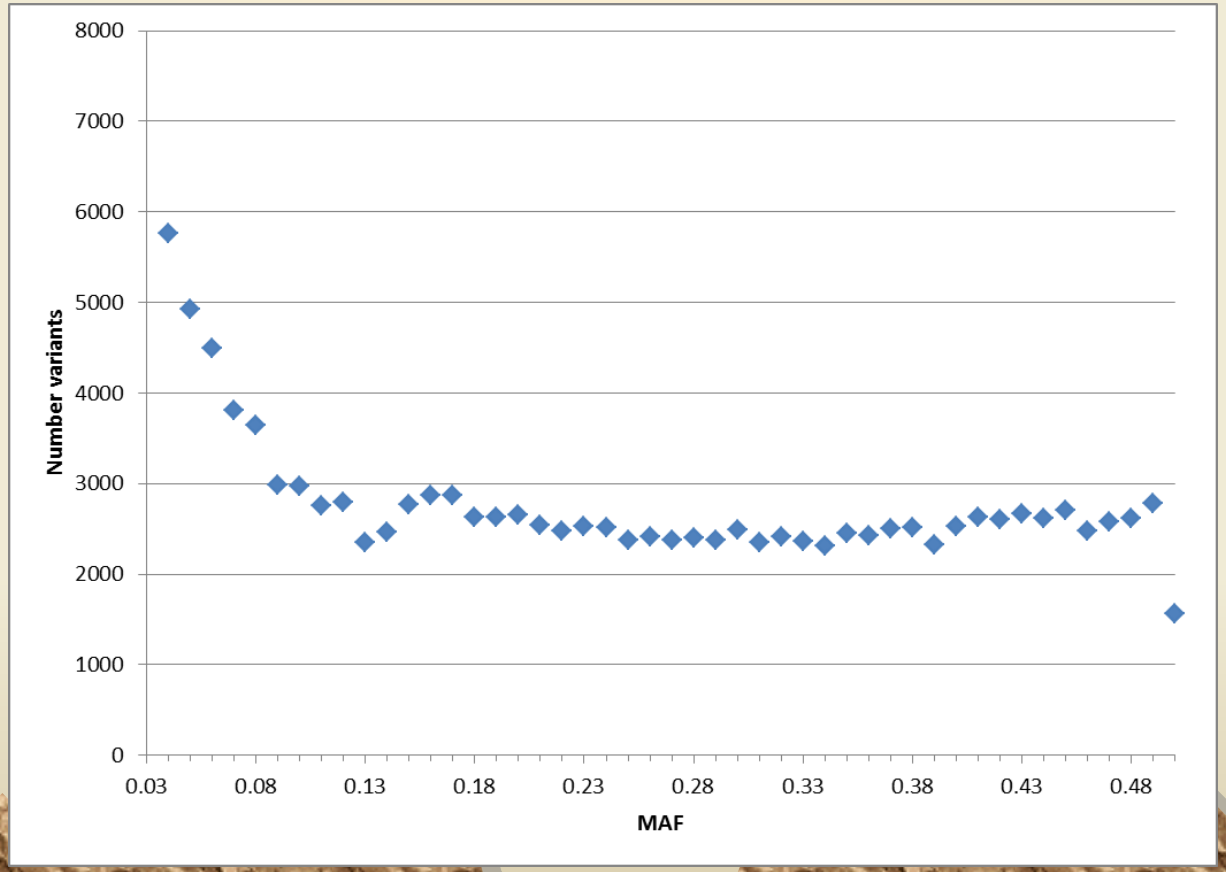
- Chip content for design was from sequences from Mizzou or 1000 bulls project
- Sources of variants:
  - 244 *Bos taurus* genomes
  - 150 *Bos taurus* animals with RNA-Seq data
  - 1000 bulls project variant calls
- Interesting variants classified by type of mutation (non-synonymous, frameshift, premature stop, etc.)
  - "No HOM" classified as variants observed in 2 individuals and no homozygotes observed
    - Within annotation boundaries for gene (not necessarily coding)
    - Variants considered validated if observed in multiple data sources (design pools, any available chips, Affy screening array and dbSNP)
  - Some of these variants not designable (high-freq variants in flanking sequence)
- Detailed information: Jerry Taylor (taylorjerr@missouri.edu)

Variant Summary

Number Variants	Description
33,730	Imputation content
193,503	Functional content
227,233	Total variants
31,835	from HD as part of imputation content
22,183	from SNP50 as part of imputation content (subset of HD)
6,395	from HD as part of functional content
443	from SNP50 as part of functional content (subset of HD)
38,230	Total HD
22,626	Total SNP50 (subset of HD)
22,298	NS Sift deleterious
48,994	NS Sift tolerated
49,627	NS no sift prediction
120,919	Total Non-synonymous (NS) AA substitutions
1,265	Frameshift indels
585	In-Frame indels
20,402	UTR
1,573	Ensembl ncRNA (snoRNA, miRNA, snRNA, rRNA,Mt_tRNA, Mt_rRNA)
4,081	Conserved non-coding elements
6,378	Splice (not mutually exclusive)

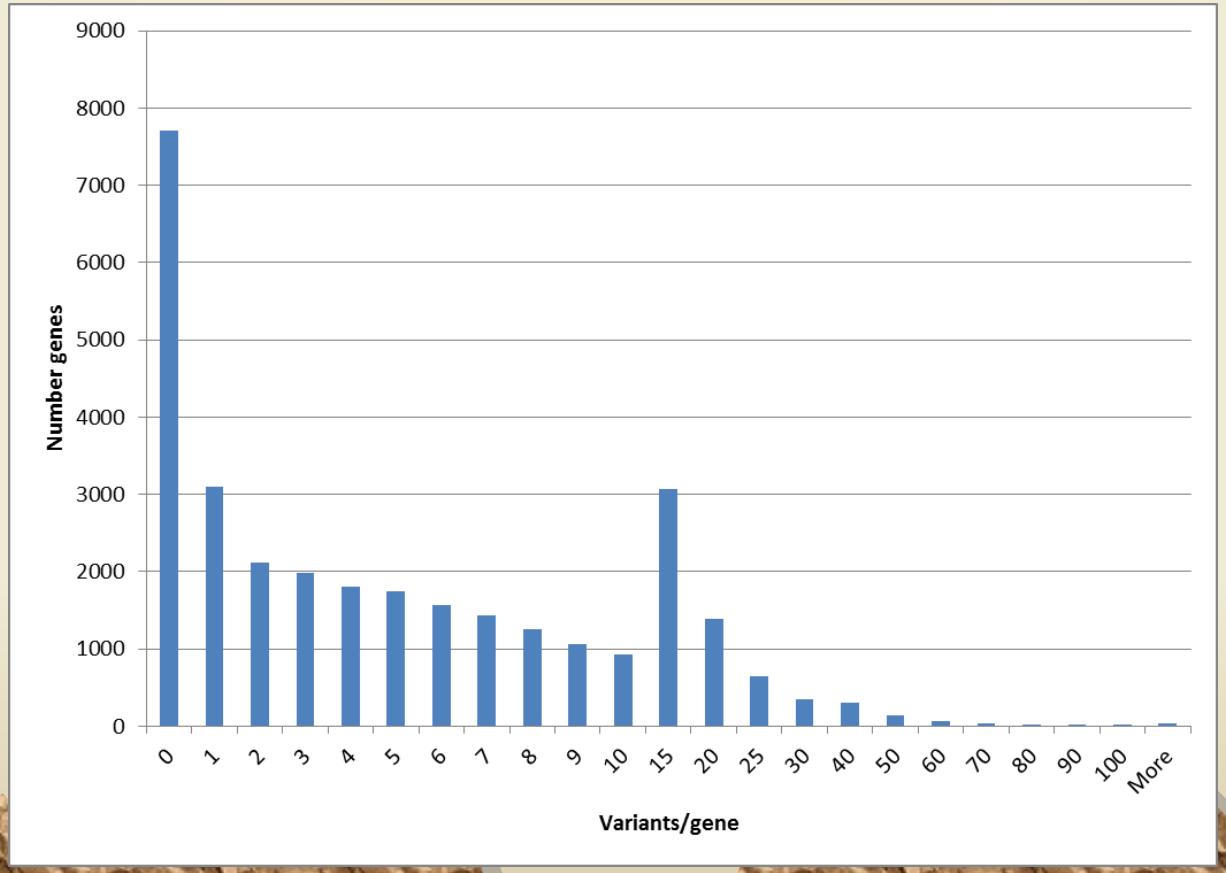
Distribution of MAF

Biased towards low frequency variants



Number of Genes Represented

Most genes at least 1 variant



23,059	Genes with at least 1 variant
7,714	Genes with no variant

General Design of GGP F250 Assay

- 220K variants (GGP F250)

- 24K for imputation to 50K or greater
- 196K potentially functional variants , biased towards genic regions

- In design now - available to all late Fall 2015

The anticipated cost of the assay will be about $100 per sample dependent on volume.

Inquiries can be directed to Stewart Bauck at GeneSeek (SBauck@neogen.com).

Progress to Date

- DNA sampling and phenotypic data collection (heifers)
  - 10,251 Angus heifers have been sampled
    - Complete reproductive data from 44 farms and ranches
  - Genotyping on GGP F250 will be completed in fall 2015

Progress to Date

- Develop selection indexes that support multi-trait selection, inclusive of fertility traits (MacNeil)
  - Economically rational emphases for fertility, growth, efficiency, and carcass traits upon which to base selection and mating decisions
- Development of decision support software that can incorporate information on LOF alleles and make selection decisions based on a relevant index or breeding objective (Kinghorn)
  - Called MateSel
  - Balance long-term selection gains, inbreeding, and frequency of recessive alleles or carrier to carrier matings
  - MateSel can also show you the ‘opportunity cost’ of imposing non–optimal constraints on mate
    - Selecting against affected progeny more effective than selecting against carriers

Van Eenennaam, A.L., and B. P. Kinghorn. 2014. Use of Mate Selection Software to Manage Lethal Recessive Conditions in Livestock Populations. WCGALP Vancouver, Canada. https://asas.org/docs/default-source/wcgalp-posters/408_paper_9819_manuscript_1027_0.pdf

Specific Aims (Extension and Education)

5

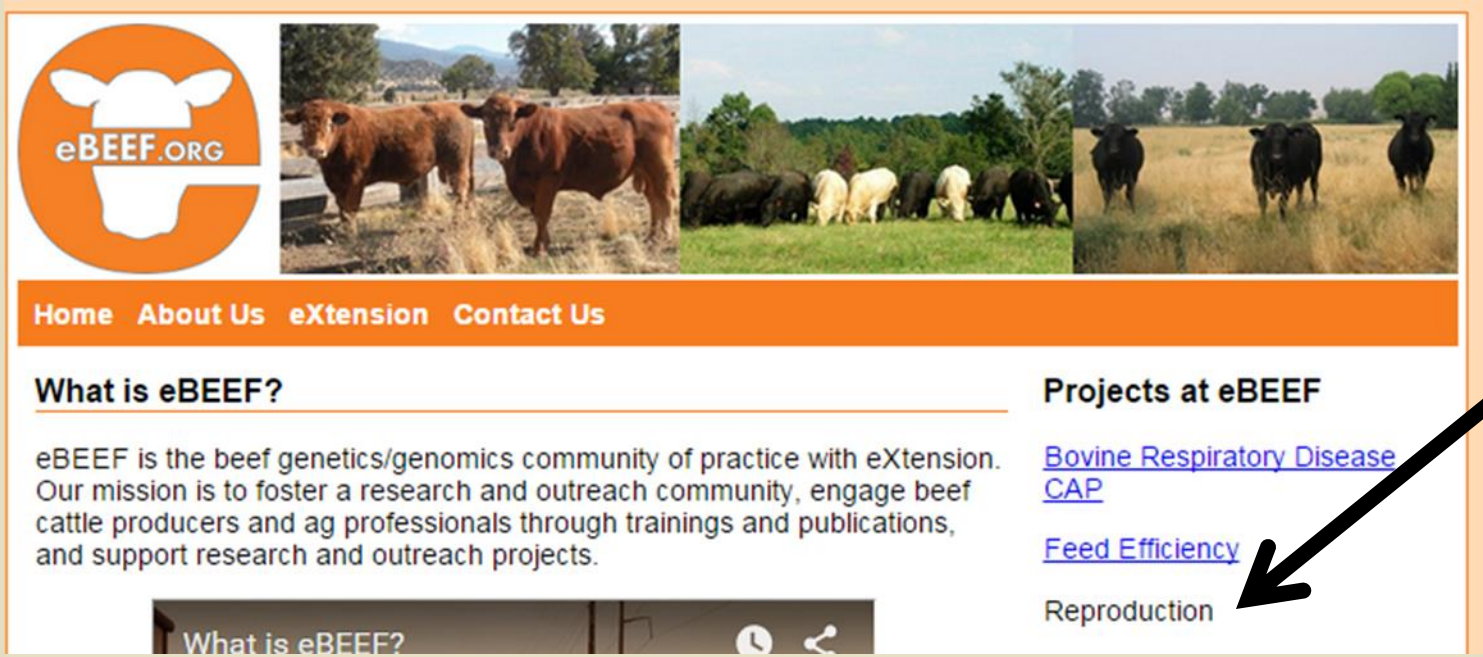
- Develop simulation exercise that demonstrates the effect of DGV for heifer and sire fertility on reproductive performance and profitability (Smith, coming 2016)

6

- Develop a web-based educational training program (Rolf, Smith and Van Eenennaam)

Educational Modules

Educational Resource Links



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