Genotypes of Cornell Dorset and Dorset Crosses Compared with Romneys for Melatonin Receptor 1a

By Christian Posbergh Cornell Undergraduate Honor Student, Dept. Animal Science

Abstract:

Sheep are known as seasonally polyestrous breeders, meaning they only breed when day length shortens in the fall. Ewes respond to these changing day lengths through melatonin receptors. However certain breeds such as Dorset, are known to be less seasonal with some individuals being able to breed year round. The *Melatonin Receptor 1a (MTNR1A)* gene was identified as a candidate gene for out of season breeding. The first studies found a SNP that was associated with a shorter time to first lambing and a shorter period between lambing using ewes managed under the Cornell STAR accelerated lambing system. The favorable allele was denoted the *M* allele and the unfavorable allele, the *m* allele. This study sought to compare allele frequencies of this polymorphism between the Cornell flock and outside flocks not selected for out of season breeding. Frequencies of the *M* allele were higher in the Cornell Flock (0.81) as opposed to outside flocks (0.59) ($p<0.002$). Using 63 genotyped Cornell ewes, having greater than one lambing record, no significant association was found between having the *M* allele and increased lambings per year, delivered per year, born alive per year, and weaned per year. This study shows that the *M* allele may not be a beneficial marker for use in flocks seeking to improve production and the ability to lamb out of season. These findings warrant future genetic research of out of season breeding.

Genotypes of Cornell Dorset and Dorset Crosses Compared with Romneys for *Melatonin Receptor 1a*

Christian Posbergh

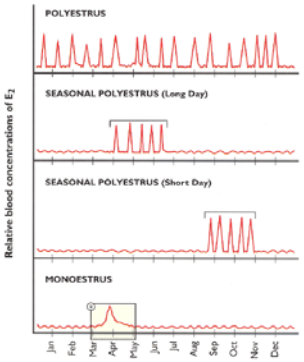
My Background

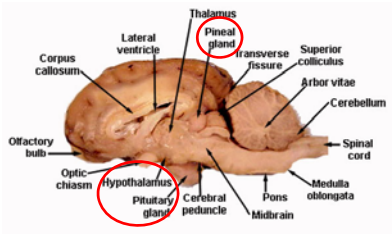
- Raised Sheep for 14 years
- Flock of Romneys & Dorsets
- District 2 Director for ARBA
- Committee Member for NSIP
- Senior Undergraduate
- Honors Thesis Project
- Genetics in Beef, Horses, Sheep & Musk Ox

Out of Season Breeding

Seasonally Polyestrous

- Many cycles but seasonally
- Most breeds enter anestrus during late spring/early summer as day length increases
- Photoperiod
- Melatonin





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Out of Season Breeding

- Occurs on 25% of breeding operations (USDA, 2014)
- Importance has increased 3x to 34%
- More sheep selected for out of season

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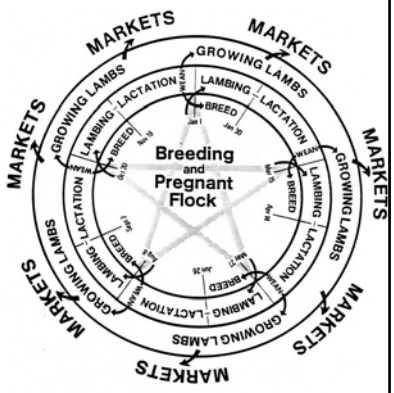
Cornell Flock

- Has selected for out of season breeding for 30 years using STAR system
- ~300 ewes
- Finnsheep
- Dorset
- Finn x Dorset



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Breeds

Dorset

- **Aseasonal**
- Meat
- Medium sized
- Mothering

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Breeds

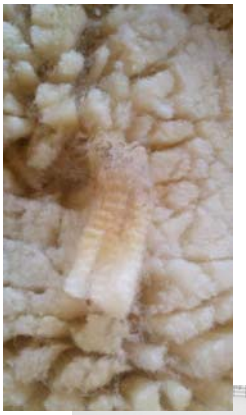
Finnsheep

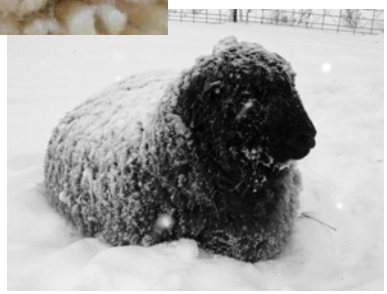
- **Multiples** & mothering
- Small-medium sized
- Growth rate & wool

Breeds

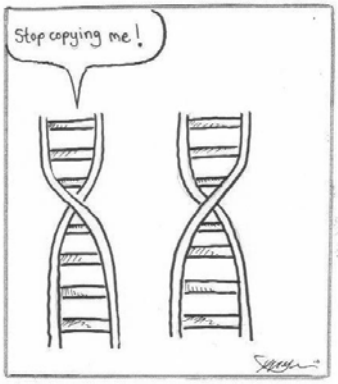
Romney

- Long wool
- Dual Purpose
- Mothering
- Temperament





Genetic Background



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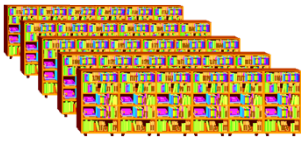
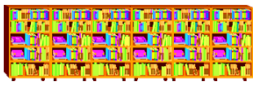
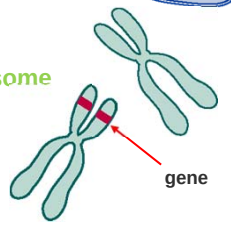
DNA hierarchy of information



library = information in every cell



1 shelf = chromosome



1 book = gene

The sentences in a book are like the sequences of nucleotide bases in DNA.

paragraph = DNA sequences

```
ATT CGG CAT CGT GGG ATC GTT
ACT AAA TCT GAG GCG GTT ACC
TAA GTT ATG GCG CCC TGT TAA
ATG GAA ACG TCT GCC CTT ATG
ACC ATT AGA ATT GGG CGG ATT
```

Words give instruction and codons specify amino acids for proteins.

words = codons

```
… ACG GGC TAA TGC ATT …
  ||| ||| ||| ||| |||
… TGC CCG ATT ACG TAA …
```

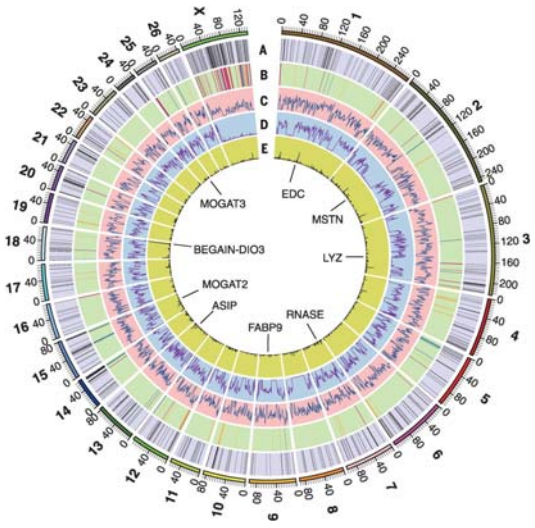
A B C D E F G H I J K L M N O P Q R S T U V W X Y Z

alphabet = DNA bases

A C T G

ANSC2210_Lecture 2

Fig. 1 The genome of sheep.(A) A total of 1097 segmental duplications (length >5 kb) in the two Texel sheep (10).



Y Jiang et al. Science 2014;344:1168-1173

Science AAAS

Published by AAAS

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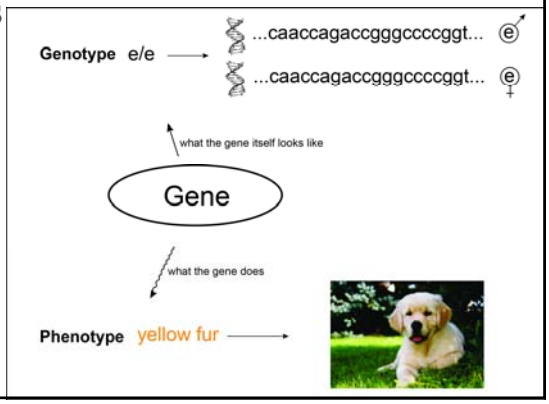
Phenotype & Genotype

- Phenotype
  - What is physically observed
- Genotype – DNA finger print
  - What the DNA says



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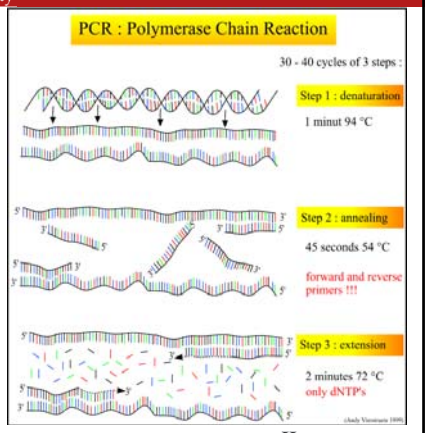
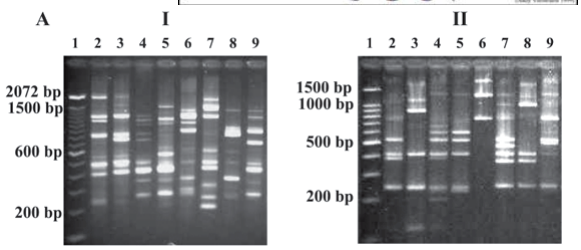
Methods

- PCR
  - Polymerase Chain Reaction
- RFLP
  - Restriction Fragment Length Polymorphism





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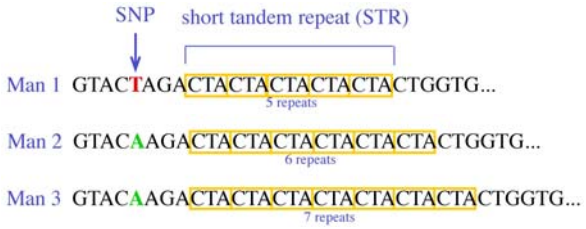
Mutation Markers

- SNPs
  - Single Nucleotide Polymorphism
- Microsatellites
  - Utilize repeats in DNA



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Genetics of Out of Season Breeding in Sheep

- 120 Microsatellites
- Found Quantitative Trait Loci (QTL) on chr 1,3,12,17,20,24
- There are many areas that affect this complex trait

Mateescu & Thonney, 2010

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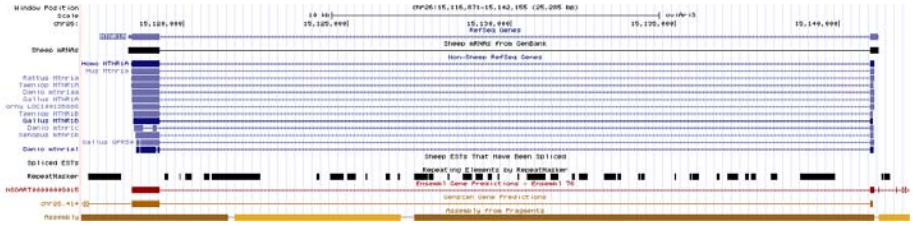
Melatonin Receptor 1a (*MTNR1A*)

- Candidate Gene
- G protein-coupled receptor which binds melatonin
- Ovine chr26



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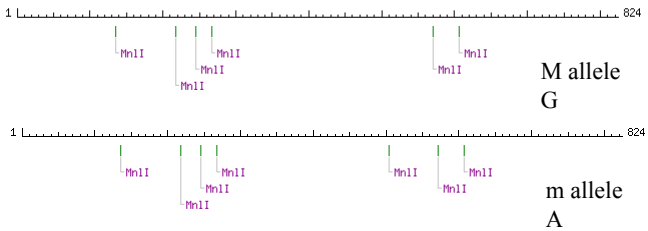
MTNR1A Polymorphism

- A → G
- 'Silent' SNP - No amino acid change
- Digested by *Mn1I* enzyme



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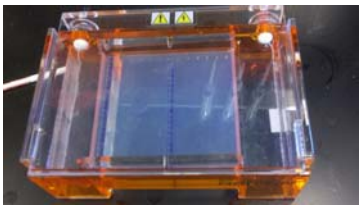
Genotyping



- Gel Electrophoresis
- *M* (G) allele – 303 bp
- *m* (A) allele – 236 & 67 bp

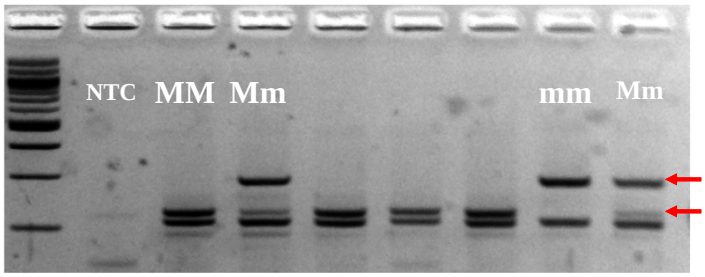


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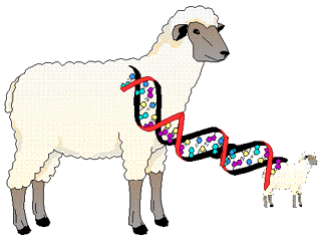
Table 3.
Effect estimates, SE, and probability > | t| for contrasts evaluating the effect of season of birth o season of 1st conception and *MnlI* and *RsaI* genotype on the number of days from birth to 1st lambing and the number of days from the 1st to the 2nd lambing

	Days to 1st lambing			Days from 1st to 2nd lambing		
Contrast[1]	Constant	SE	$P > \|t\|$	Constant	SE	$P > \|t\|$
Effect of season of birth[2] or 1st conception[3]						
ES and LS vs. AS	−140.76	58.61	0.02	89.46	53.52	0.10
ES vs. LS	30.7	77.86	0.70	−90.49	54.05	0.10
Effect of *MnlI* genotype						
mm vs. *Mm* and *MM*	135.76	70.1	0.05	123.59	59.75	0.04
Mm vs. *MM*	−78.77	51.7	0.13	19.83	46.33	0.70

Mateescu *et al.* (2009)

Present Study



Study Design

- Cornell ewes vs Romney ewes

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Study Design

- Collect Blood & Extract DNA

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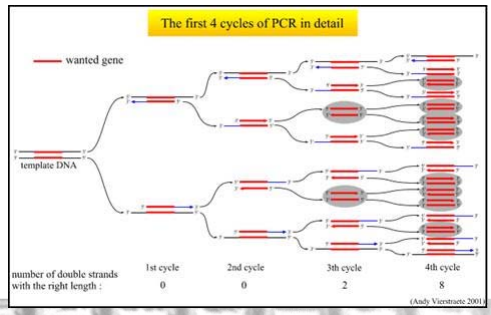
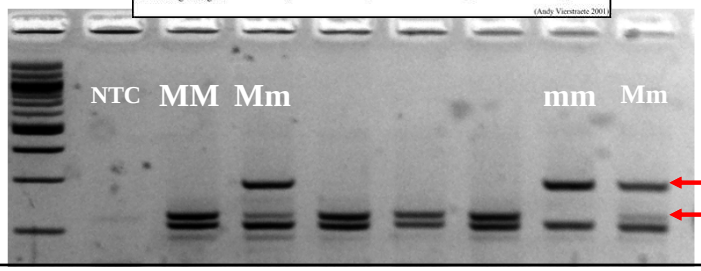
Study Design

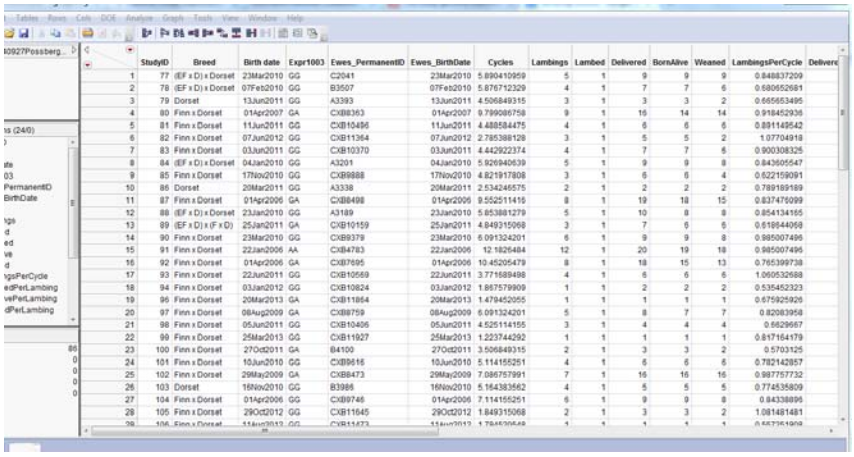
- Run PCR & RFLP for genotyping





Study Design

- Analysis of Lambing Records & Genotypes



	StudyID	Breed	Birth date	Expr1063	Ewes_PermanentID	Ewes_BirthDate	Cycles	Lambings	Lambed	Delivered	BornAlive	Weaned	LambingsPerCycle	Delivere
1	77	(EF x D) x Dorset	23Mar2010	GG	C2041	23Mar2010	5.890410959	5	1	9	9	9	0.848837209	
2	78	(EF x D) x Dorset	07Feb2010	GG	B3507	07Feb2010	5.876712329	4	1	7	7	6	0.680652681	
3	79	Dorset	13Jun2011	GG	A3393	13Jun2011	4.506849315	3	1	3	3	2	0.665653495	
4	80	Finn x Dorset	01Apr2007	GA	CXB8363	01Apr2007	9.799086758	9	1	16	14	14	0.918452936	
5	81	Finn x Dorset	11Jun2011	GG	CXB10496	11Jun2011	4.488584475	4	1	6	6	6	0.891149542	
6	82	Finn x Dorset	07Jun2012	GG	CXB11364	07Jun2012	2.785388128	3	1	5	5	2	1.07704918	
7	83	Finn x Dorset	03Jun2011	GG	CXB10370	03Jun2011	4.442922374	4	1	7	7	6	0.900308325	
8	84	(EF x D) x Dorset	04Jan2010	GG	A3201	04Jan2010	5.926940639	5	1	9	9	8	0.843605547	
9	85	Finn x Dorset	17Nov2010	GG	CXB9888	17Nov2010	4.821917808	3	1	6	6	4	0.622159091	
10	86	Dorset	20Mar2011	GG	A3338	20Mar2011	2.534246575	2	1	2	2	2	0.789189189	
11	87	Finn x Dorset	01Apr2006	GA	CXB6498	01Apr2006	9.552511416	8	1	19	18	15	0.837475099	
12	88	(EF x D) x Dorset	23Jan2010	GG	A3189	23Jan2010	5.853881279	5	1	10	8	8	0.854134165	
13	89	(EF x D) x (F x D)	25Jan2011	GA	CXB10159	25Jan2011	4.849315068	3	1	7	5	5	0.618644068	
14	90	Finn x Dorset	23Mar2010	GG	CXB9379	23Mar2010	6.091324201	6	1	9	9	8	0.985007496	
15	91	Finn x Dorset	22Jan2006	AA	CXB4783	22Jan2006	12.1826484	12	1	20	19	18	0.985007496	
16	92	Finn x Dorset	01Apr2006	GA	CXB7695	01Apr2006	10.45205479	8	1	18	15	13	0.765399738	
17	93	Finn x Dorset	22Jun2011	GG	CXB10569	22Jun2011	3.771689498	4	1	6	6	6	1.060532688	
18	94	Finn x Dorset	03Jan2012	GG	CXB10824	03Jan2012	1.867579909	1	1	2	2	2	0.535452323	
19	96	Finn x Dorset	20Mar2013	GA	CXB11864	20Mar2013	1.479452055	1	1	1	1	1	0.675925926	
20	97	Finn x Dorset	08Aug2009	GA	CXB8759	08Aug2009	6.091324201	5	1	8	7	7	0.82083958	
21	98	Finn x Dorset	05Jun2011	GG	CXB10406	05Jun2011	4.525114155	3	1	4	4	4	0.6629667	
22	99	Finn x Dorset	25Mar2013	GG	CXB11927	25Mar2013	1.223744292	1	1	1	1	1	0.817164179	
23	100	Finn x Dorset	27Oct2011	GA	B4100	27Oct2011	3.506849315	2	1	3	3	2	0.5703125	
24	101	Finn x Dorset	10Jun2010	GG	CXB9616	10Jun2010	5.114155251	4	1	6	6	5	0.782142857	
25	102	Finn x Dorset	29May2009	GA	CXB8473	29May2009	7.086757991	7	1	16	16	16	0.987757732	
26	103	Dorset	16Nov2010	GG	B3986	16Nov2010	5.164383562	4	1	5	5	5	0.774535809	
27	104	Finn x Dorset	01Apr2006	GG	CXB9746	01Apr2006	7.114155251	6	1	9	9	8	0.84338896	
28	105	Finn x Dorset	29Oct2012	GG	CXB11645	29Oct2012	1.849315068	2	1	3	3	2	1.081481481	

Actual Samples Collected

- Outside Flocks
  - Romney
    - 54 total, 49 ewes, 5 rams
  - Dorset
    - 3 total, 3 ewes
- Cornell Flock
  - Dorset
    - 23 total, 11 rams, 12 ewes
  - Finn x Dorset
    - 66 total, 20 rams, 46 ewes
  - (EF x D) x Dorset
    - 8 total, 8 ewes
  - 1/8EF 7/8Dorset
    - 4 total, 4 rams
  - Finnsheep
    - 3 total, 3 rams

Genetic analysis

$$p^2 + 2pq + q^2 = 1$$

Hardy-Weinberg Equation

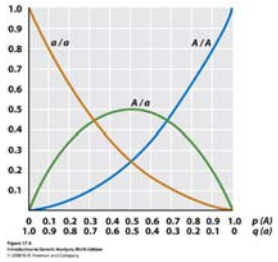
$$p = 1$$

Easy-Weinberg Equation

science.memebase.com

No selection

- Under no selection & random mating, allele frequencies shouldn't change
- Under selection allele frequencies will change
- By testing observed vs expected can determine if it's due to chance



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Other Breed Frequencies

Breed	Sample	Allele Frequency *M*	*m*	Source	Country
Dorset	116	0.64	0.36	Mateescu et al. (2009)	USA (Cornell)
Chios	48	0.90	0.10	Şeker et al. (2011)	Turkey
White Karaman	40	0.80	0.20		
Awassi	50	0.84	0.16		
Gotland	28	0.87	0.13	Falk (2013)	Sweden
Swedish Finewool	30	0.82	0.18		
Roslag	32	0.71	0.29		
Rasa Aragonesa	80	0.87	0.13	Martínez-Royo et al. (2012)	Spain
Chokla	101	0.88	0.12	Saxena et al. (2014)	India
Sarda	600	0.77	0.23	Carcangiu et al. (2011)	Italy
Zel	100	0.65	0.35	Moradi et al. (2014)	Iran
Naeini	50	0.71	0.29		
Karakul	100	0.79	0.21	Shahroudi (2006)	Iran
Small Tail Han	106	0.75	0.25	Chu et al. (2003)	China
Romney	54	0.59	0.41	Posbergh (unpublished)	USA
Dorset	23	0.74	0.26		
Finn x Dorset	66	0.78	0.22		

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Results

		Genotype Frequency			Allele Frequency	
Flock	Size	*MM* (GG)	*Mm* (GA)	*Mm* (AA)	*M* (G)	*m* (A)
Cornell	81	53	25	3	0.81	0.19
Romney	54	20	24	10	0.59	0.41

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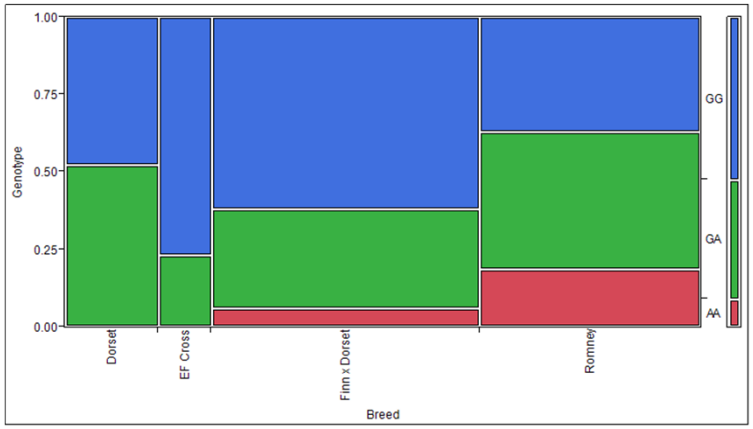
Results – Genotype by Breed



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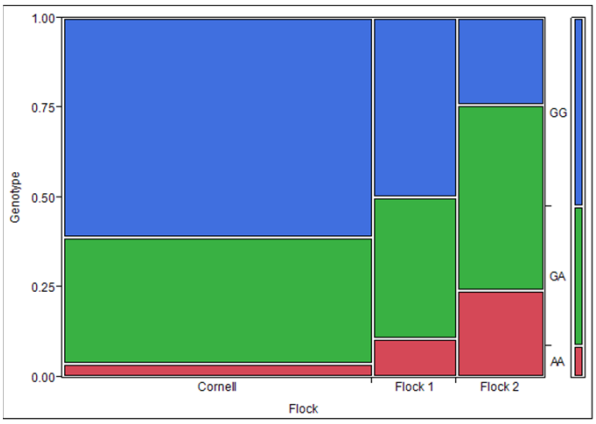
Results – Genotype by Flock



# of Animals
Cornell – 103
Flock 1 – 28
Flock 2 – 29

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Results – Cornell Lambing Record Analysis

- Records on all Cornell Ewes
  - 63 genotyped ewes used for analysis
- Lambing records used
  - Lambings per year
  - Delivered per year
  - Born Alive per year
  - Weaned per year

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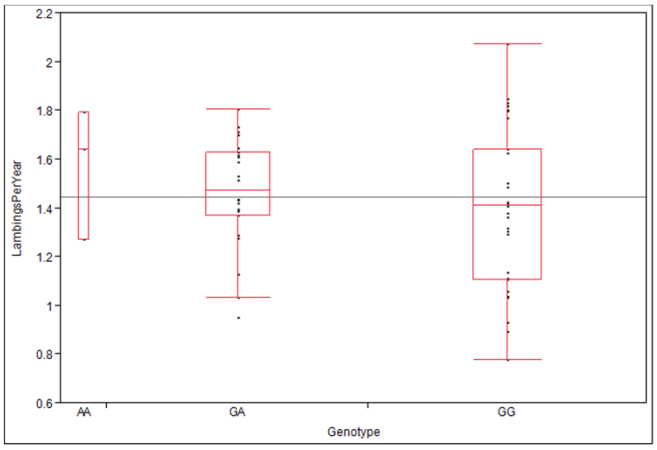
Results – Cornell Lambing Record Analysis Lambings per year



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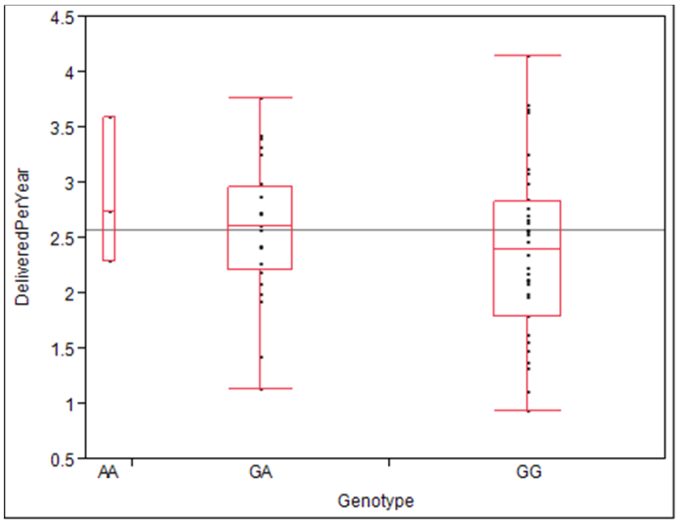
Results – Cornell Lambing Record Analysis Delivered per year



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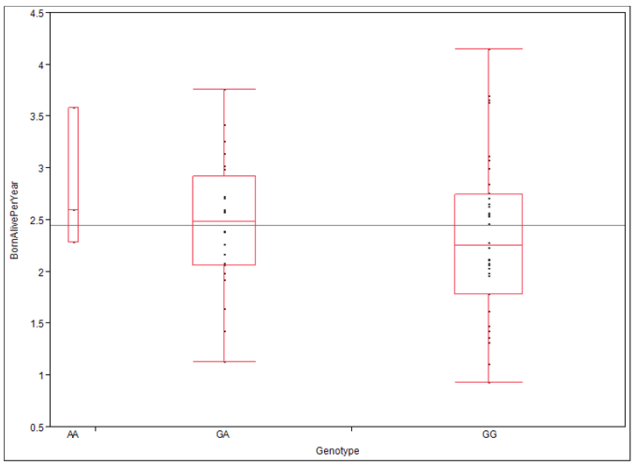
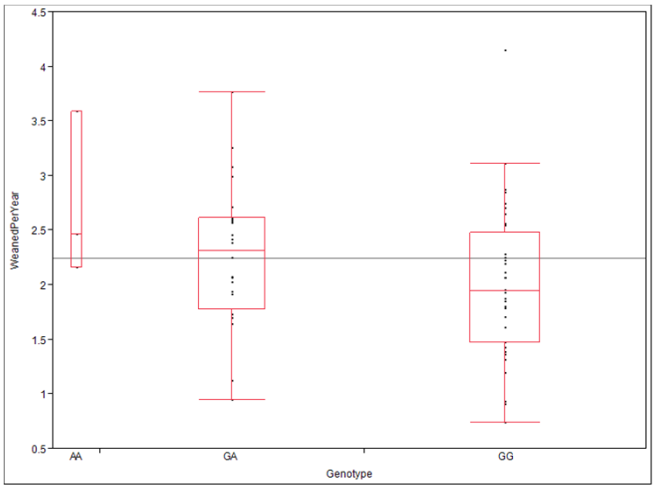
Results – Cornell Lambing Record Analysis Born Alive per year



Results – Cornell Lambing Record Analysis
Weaned per year



Conclusions & Future Directions

Is this where we are headed?

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Conclusions

- No significant association with production traits
- However significant differences between breed & flocks, indicating it may be under selection
- Remember: 1 SNP in 1 gene out of 10,000 genes in the genome
  - Complex trait likely has many genetic components
- Good basis for future research

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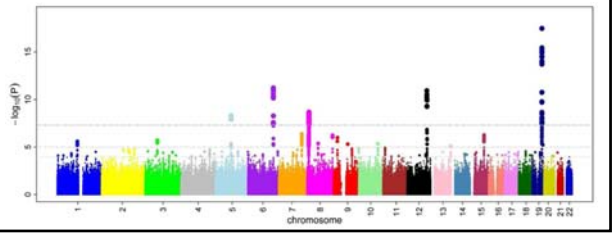
Future Directions

- Use of new technology such as the 50K or 600K SNP chip
- Test in other flocks selecting for aseasonality
- Challenge breed animals for accurate phenotyping
- MORE DATA!



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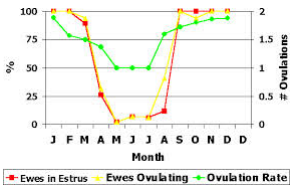
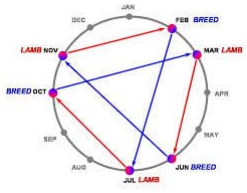
Applications

- If validated more, could be used as a marker to assist selection
- Utilized by breeders wishing to breed out of season or accelerate lambing naturally





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Acknowledgements

- Dr. Michael Thonney
- Dr. Samantha Brooks
- Dr. Heather Huson

- Natasha Pettifor
- Ann Staiger
- Katherine Churchill
- Rachel Murphy

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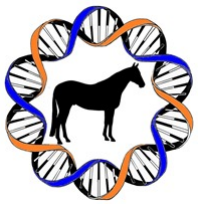
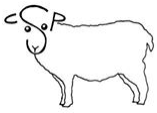


Brooks Equine Genetics Lab



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Questions?