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Genome-wide association analyses for loci controlling boar taint

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**Danish Slaughterhouses*

SABRE CUTTING EDGE GENOMICS FOR SUSTAINABLE ANIMAL BREEDING

ROSLIN

Aim

- to use a genomics approach to study the genetic control of boar taint in entire male pigs

Boar taint

Boar taint

- Strong urine / faecal smell
- ~10% intact males

Solutions

- Castrate
- Slaughter younger

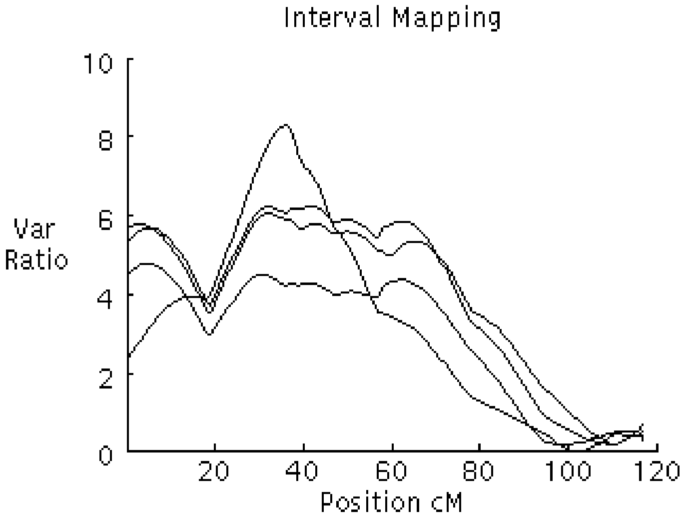
Impacts

- Animal welfare
- Product quality
  - taste, fat/lean
- Sustainability
  - feed efficiency
  - environmental
  - economic

Genetics, genome scan

- ☐ Large White / Meishan
- ☐ No evidence for major gene
- ☐ QTL for taint
  - skatole, indole
  - androstenone

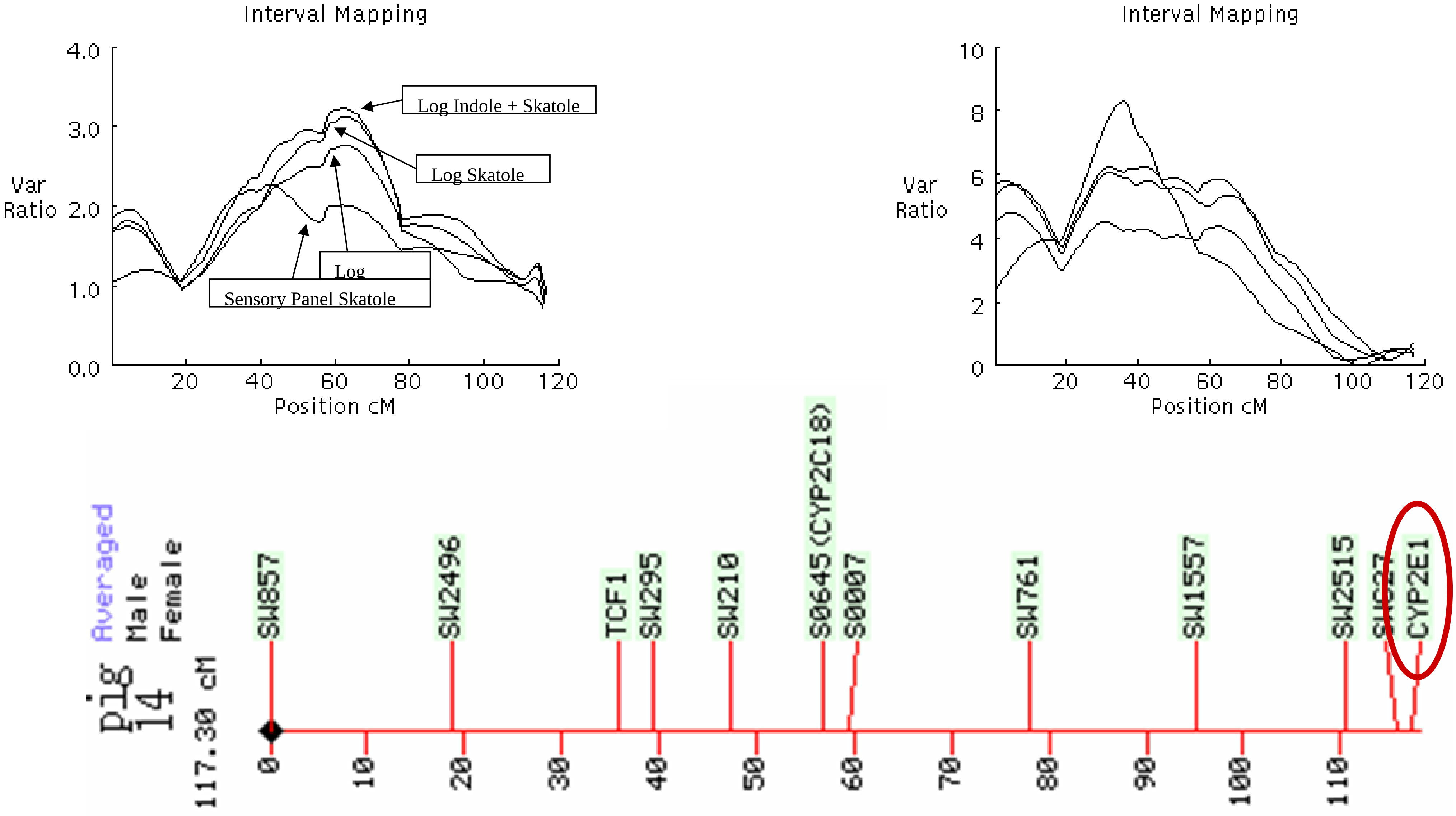
- ☐ Are there plausible candidate genes?



Lee *et al*., 2005 *Anim Genet* 36: 14

Cytochrome P450 2E1 - *CYP2E1*

- ☐ Physiological candidate gene
- ☐ Comparative positional candidate gene?
- ☐ Mapped to SSC14
- ☐ Isolate genomic clone (BAC)
- ☐ Develop genetic markers
- ☐ Add to linkage / QTL map
- ☐ Scan promoter for polymorphism



SABRE

CUTTING EDGE GENOMICS FOR SUSTAINABLE ANIMAL BREEDING

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Sabre WP9: Product Quality (Boar taint)

- Perform genome wide scan to confirm known QTL and identify new QTL
- Identify new candidate genes for skatole and androstenone by comparative genome-wide expression analysis and comparative proteomic studies
- Integrate mapping, expression and proteomic data, target causative genes and identify predictive SNPs
- Confirm SNPs in experimental and commercial populations

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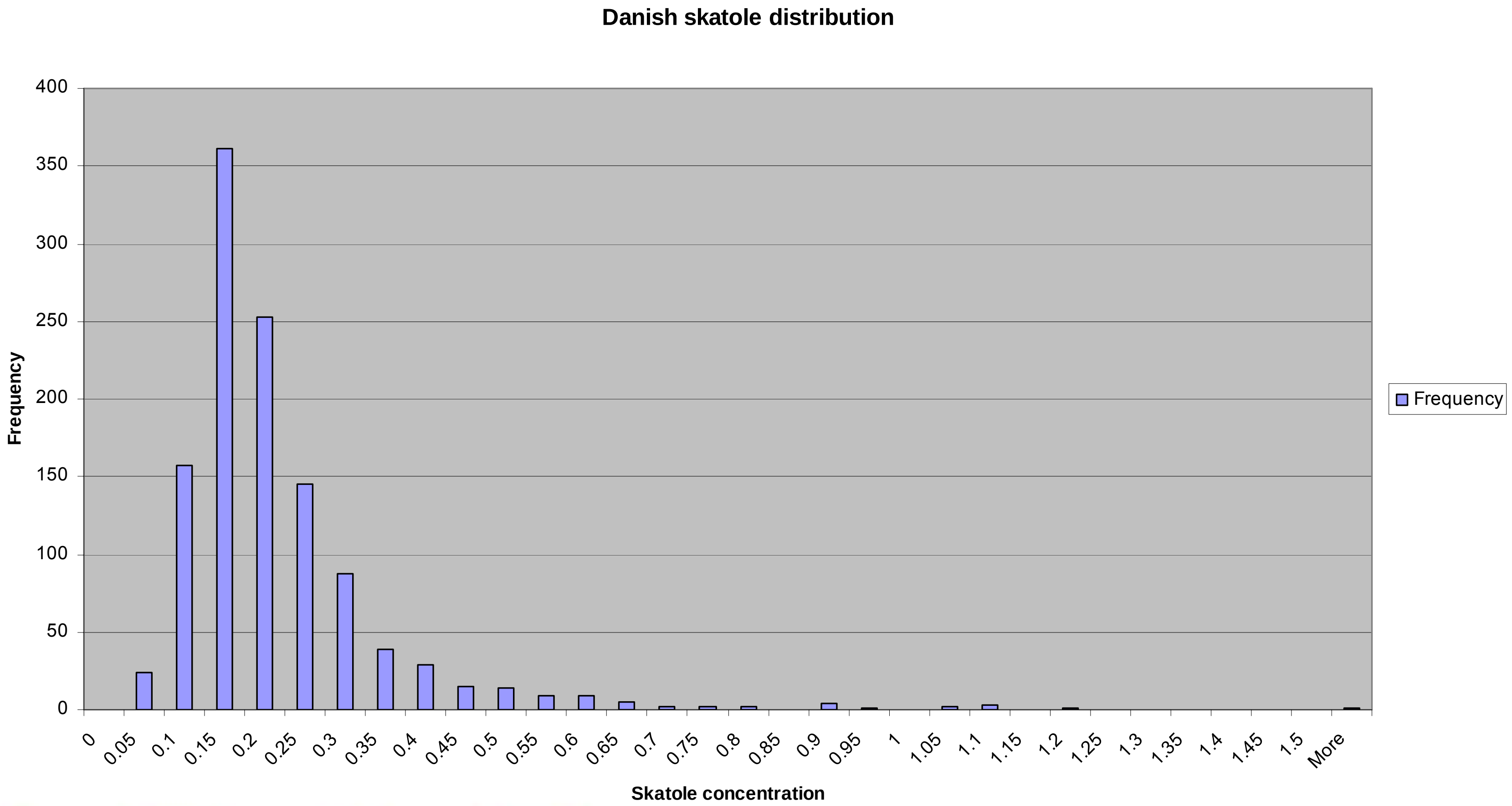
Genome scan - population

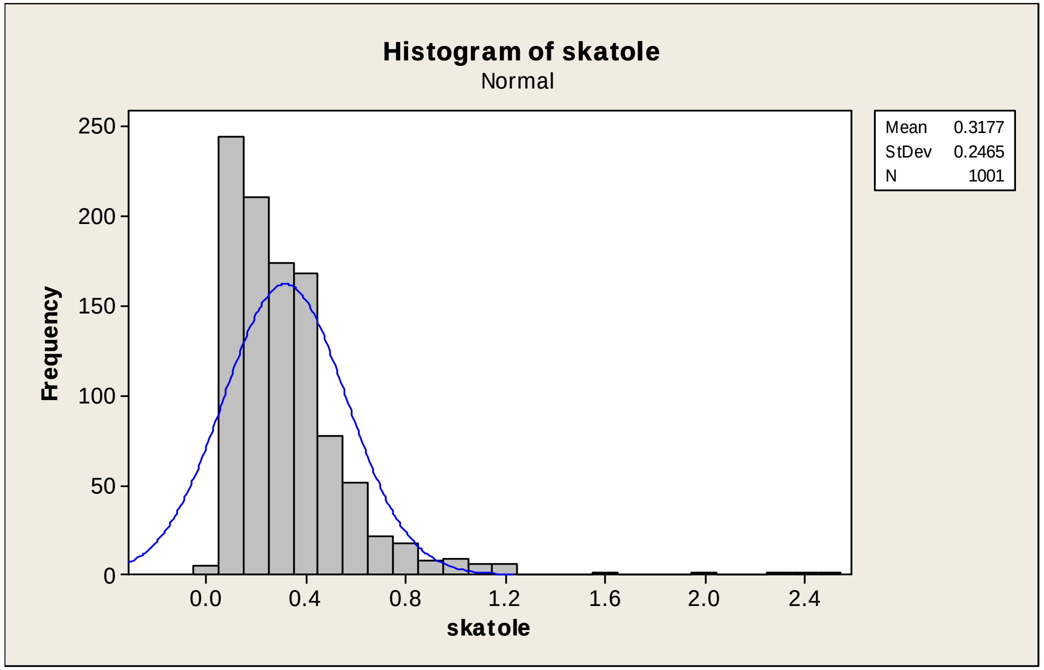
- Danish Landrace
- Danske Slagterier in-line skatole testing
- ~6,000 samples
  - select full sib pairs
  - 500 high skatole
  - 500 low skatole
- Full phenotypes – growth, fat, pedigree
- Androstenone levels assayed - Norway

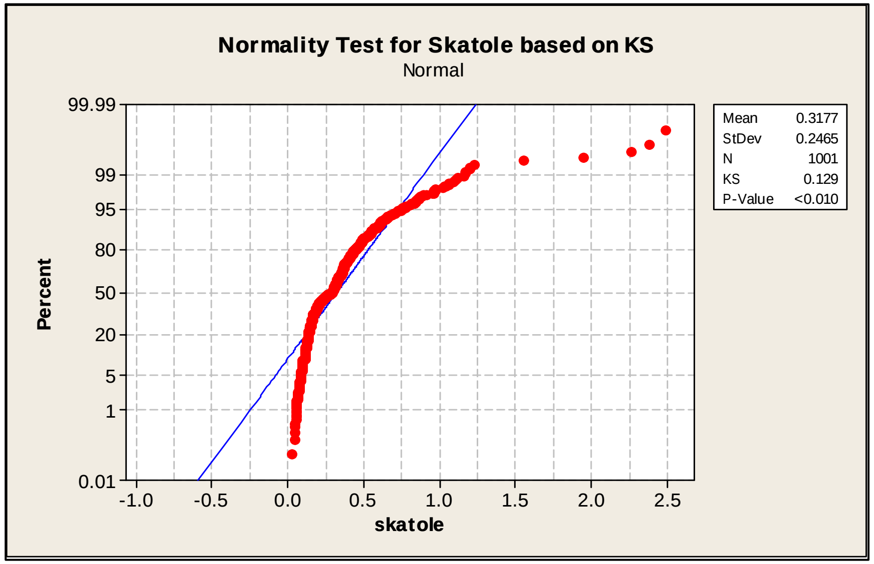
Taint data

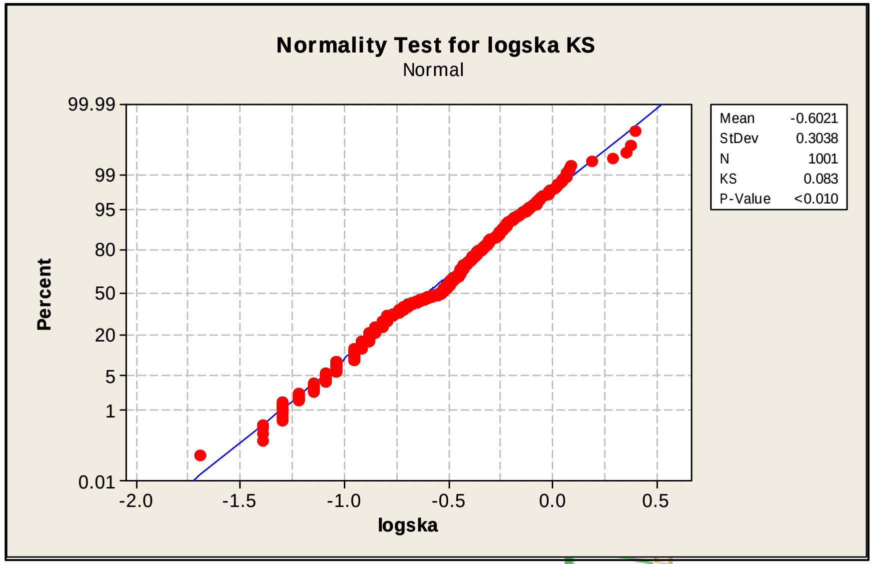
	Number of animals	Mean (SD)	Range (min-max)
Skatole (µg/g) (unselected)	6178	0.20 (0.14)	0.02 – 2.86
Skatole (µg/g) (selected)	1001	0.32 (0.25)	0.02 – 2.49
Androstenone (selected)	1002	1.04 (0.97)	0.0 – 10.62

Danish Landrace samples




Histogram of skatole
Normal


Normality Test for Skatole based on KS
Normal


Normality Test for logska KS
Normal

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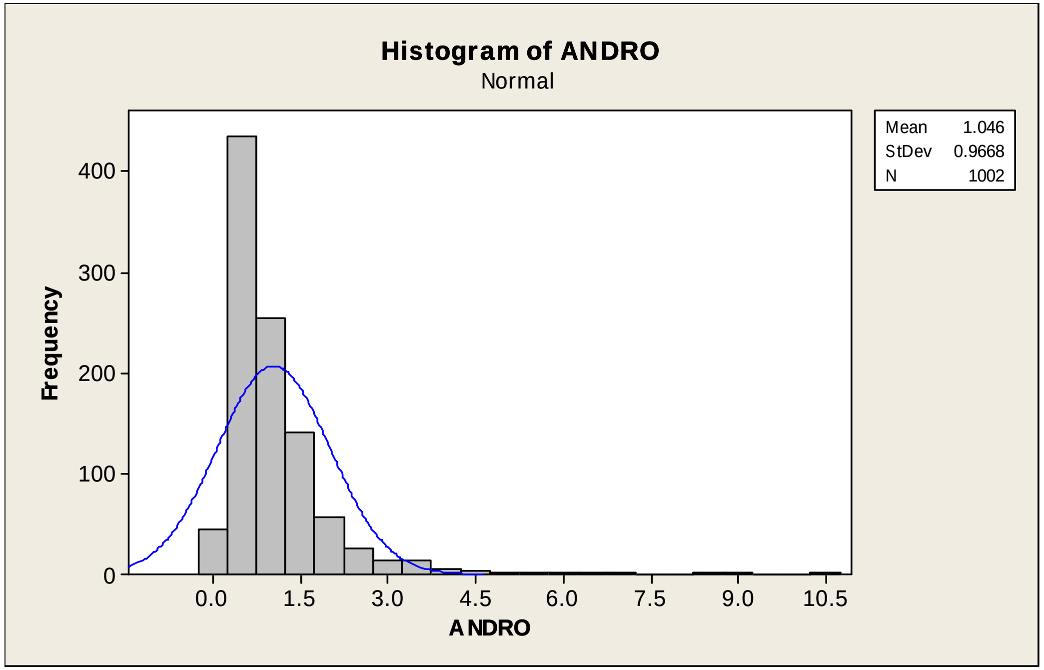
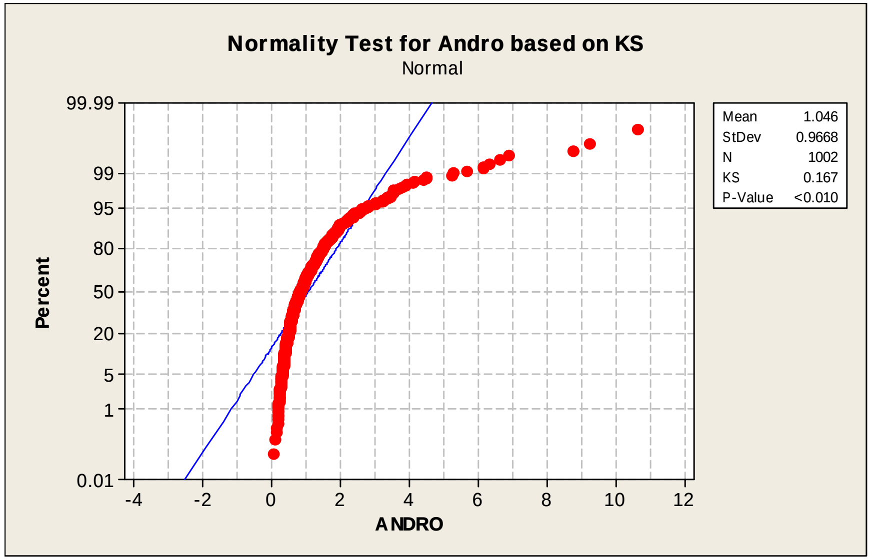
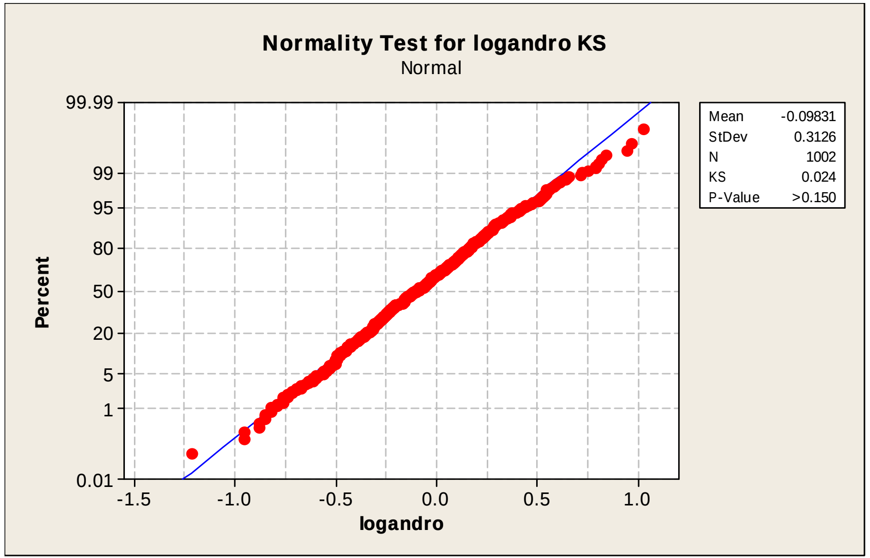

Histogram of ANDRO
Normal
Frequency
0
100
200
300
400
0.0
1.5
3.0
4.5
6.0
7.5
9.0
10.5
ANDRO
Mean 1.046
StDev 0.9668
N 1002


Normality Test for Andro based on KS
Normal
Percent
99.99
99
95
80
50
20
5
1
0.01
-4
-2
0
2
4
6
8
10
12
ANDRO
Mean 1.046
StDev 0.9668
N 1002
KS 0.167
P-Value <0.010


Normality Test for logandro KS
Normal
Percent
99.99
99
95
80
50
20
5
1
0.01
-1.5
-1.0
-0.5
0.0
0.5
1.0
logandro
Mean -0.09831
StDev 0.3126
N 1002
KS 0.024
P-Value >0.150

7K SNP chip

- Candidate SNPs
  - 1,635 (23.2%) targeted re-sequencing of BES STS (Roslin/Sanger)
  - 2,695 (38.3%) targeted re-sequencing of cDNA (Aarhus / UMB)
  - 2,712 (38.5%) *in silico* mining EST data (Aarhus)
  - 7,042 submitted for design
  - 6,523 Illumina iSelect / Infinium II assays

Data analyses

- Identification of fixed effects
  - Step-wise regression (Minitab 2006)
  - Slaughter week, herd, age, year_born,….
- Estimation of heritabilities and genetic correlations
  - Univariate and multivariate animal models ASREML (Gilmour *et al*., 2007)
- Association analyses
  - GenABEL (Aulchenko *et al*., 2007)

	Androstenone	Skatole	Unselected Skatole
Androstenone	0.56(0.10)		
Skatole		0.04-0.56 (0.03-0.10)	
Unselected Skatole			0.35(0.06)

Heritability (on diagonal), phenotypic (upper diagonal) and genetic correlation estimates for androstenone, skatole and unselected skatole.

	Androstenone	Skatole	Unselected Skatole
Androstenone		0.35-0.37 (0.03-0.03)	0.29 (0.03)
Skatole			
Unselected Skatole			

Heritability (on diagonal), **phenotypic (above diagonal)** and genetic **correlation estimates** for androstenone, skatole and unselected skatole.

	Androstenone	Skatole	Unselected Skatole
Androstenone			
Skatole	0.29-0.92 (NA)		NA
Unselected Skatole	0.42(0.11)	NA	

Heritability (on diagonal), phenotypic (upper diagonal) and **genetic correlation** estimates for androstenone, skatole and unselected skatole.

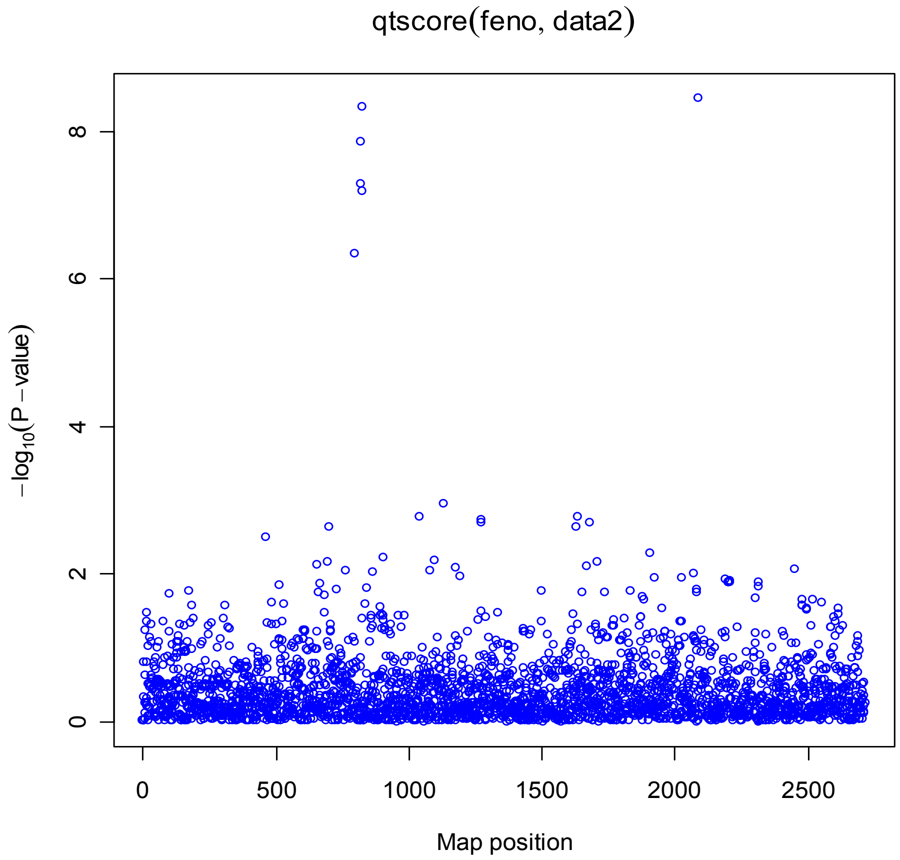
	Androstenone	Skatole	Unselected Skatole
Androstenone	0.56(0.10)	0.35-0.37 (0.03-0.03)	0.29(0.03)
Skatole	0.29-0.92 (NA)	0.04-0.56 (0.03-0.10)	NA
Unselected Skatole	0.42(0.11)	NA	0.35(0.06)

Heritability (on diagonal), phenotypic (upper diagonal) and genetic correlation estimates for androstenone, skatole and unselected skatole.

Data quality control

- Remove
  - Markers with very low MAF (< 0.02)
  - Markers with call rates < 0.95
  - Individuals with call rates < 0.95
  - Individuals with high autosomal heterozygosities
  - Individuals with high levels of homozygosity
- Number of markers 2753 (was 6523)
- Number of animals 884 (was 993)

TOP 3 MARKERS based on SIGNIFICANCE LEVEL RANKINGS (QC)



MARKER	P-VAL
stSG1354670_307	1E-10
Chr14_CYP2E1_3	1E-10
Chr14_CYP2E1_2	3E-10

TOP 3 MARKERS based on EXPERIMENT WISE RANKINGS (QC)

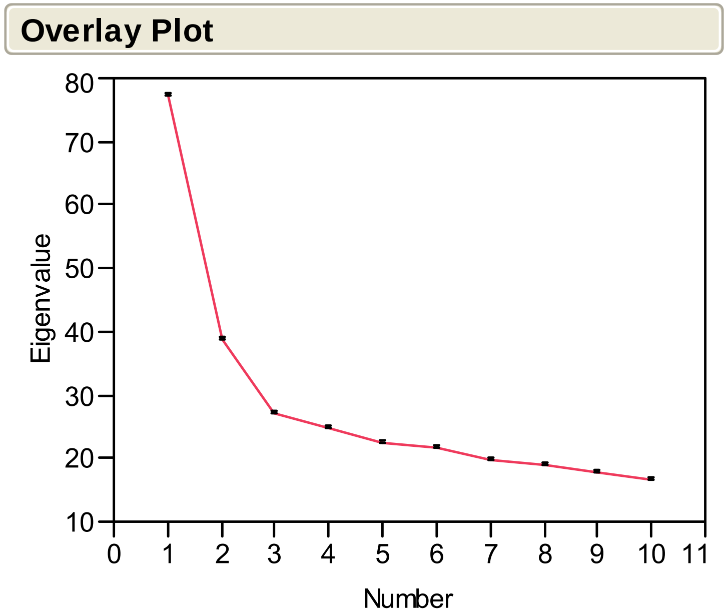
MARKER	P-VAL
Chr14_CYP2E1_1	9.999e-05
Chr14_CYP2E1_2	9.999e-05
Chr14_CYP2E1_3	9.999e-05

CYP2E1

Further tests

- Include principal components for stratification
  - 3 PCP included in model

Marker	ChiSq	P-value
stSG1354670_307	16.393	5.15E-05
Chr14_CYP2E1_3	16.02625	6.25E-05
Chr14_CYP2E1_1	15.90172	6.67E-05
Chr14_CYP2E1_4	15.26146	9.36E-05
_14133_TXNL2_11514_5	15.00728	1.07E-04
Chr14_CYP2E1_2	14.25959	1.59E-04



Further tests

- Analyze as Case-Control
  - Discordant sib-pair tests using allele counting
  - Extensive permutations

	All Alleles	DSP allele count
CYP2E	18.71(4e-05)	27.48(0/100000)
TXNL	17.92(0/100000)	24.60(0/100000)
STSG	18.80(0/100000)	32.13(0/100000)

Linking androstenone and skatole

- Correlation between androstenone and skatole
    - Inconsistent
- Bristol work suggests mechanisms
    - Androstenone inhibits CYP2E1 expression
- No effect of CYP2E1 on androstenone in current study

Future work

- Complete genome scan analyses
  - Androstenone
  - Different models
- Characterisation of candidate genes
  - CYP2E1,….
- Validation in other populations / samples
- Fine mapping / genome scan with 60K SNPs

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