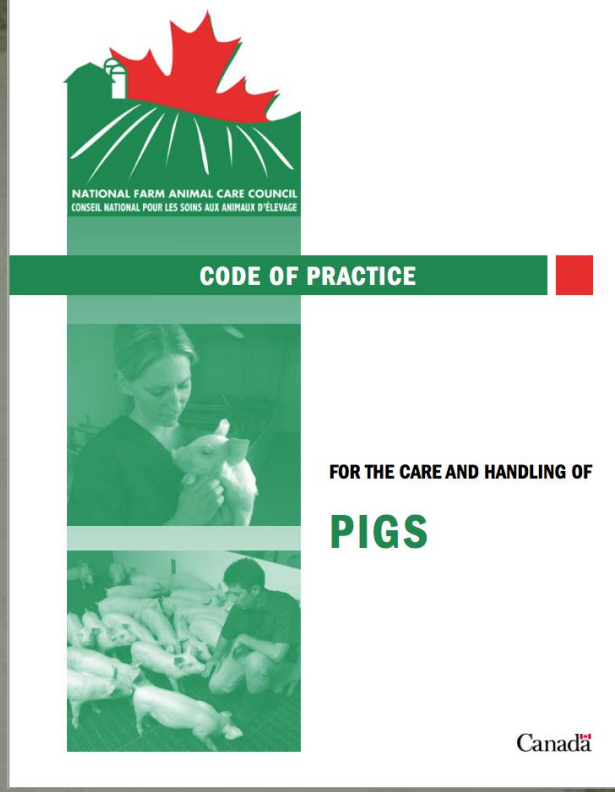
Using genetic markers to select Canadian Duroc sires for lower boar taint levels in commercial hogs

M. Jafarikia[1,2], L. Maignel[1], F. Fortin[3], S. Wyss[1], W. Van Berkel[4], D. Cohoe[5], F. Schenkel[2], J. Squires[2], B. Sullivan[1]

[1]Canadian Centre for Swine Improvement (CCSI)
[2]Centre for Genetic Improvement of Livestock (CGIL), University of Guelph
[3]*Centre de développement du porc du Québec* (CDPQ)
[4]Western Swine Association Testing (WSTA)
[5]Ontario Swine Improvement (OSI)

Boar Taint

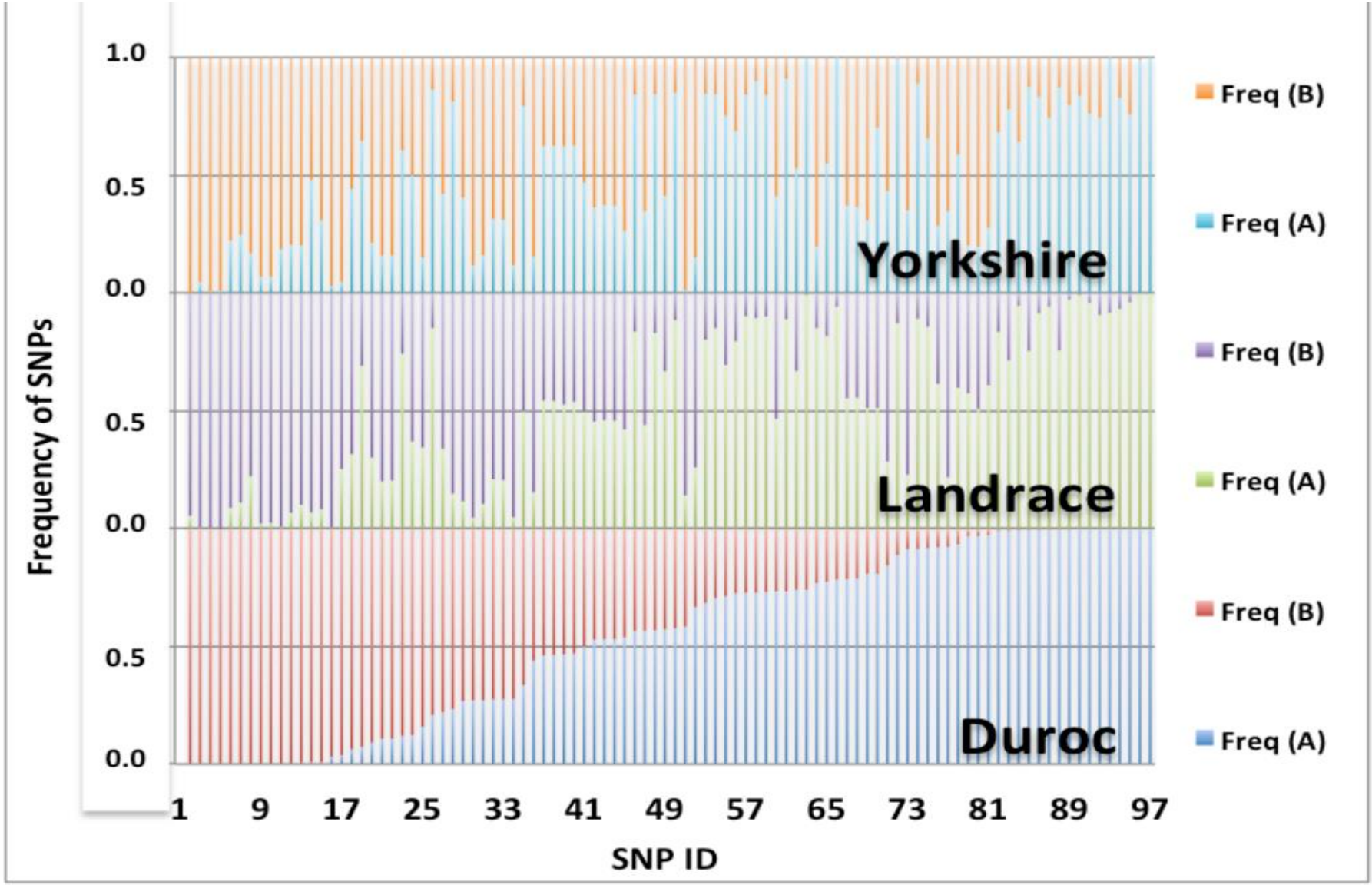
- An unpleasant odour and flavour from intact boars meat when cooked
- Solution in Canada: Castration
  - Male piglets are just a few days old
  - Pain control (analgesics)
    - Recommended for all ages (mandatory after 10 days)
    - Mandatory for all ages effective July 1, 2016
  - Recommended practices include
    - Consider alternatives to surgical castration
    - Consider marketing intact males
  - Potential negative side effects on piglet health, survival and productivity

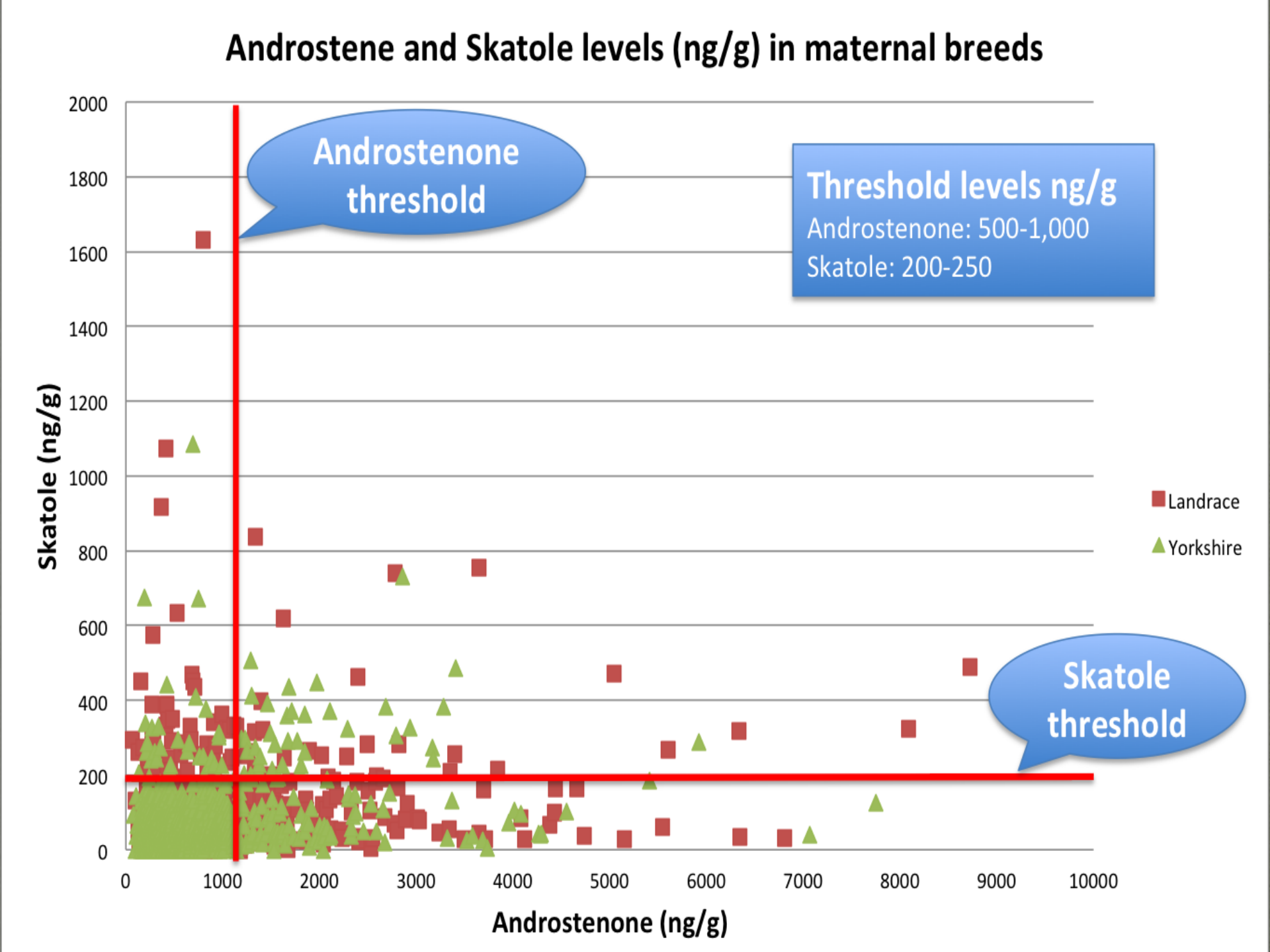


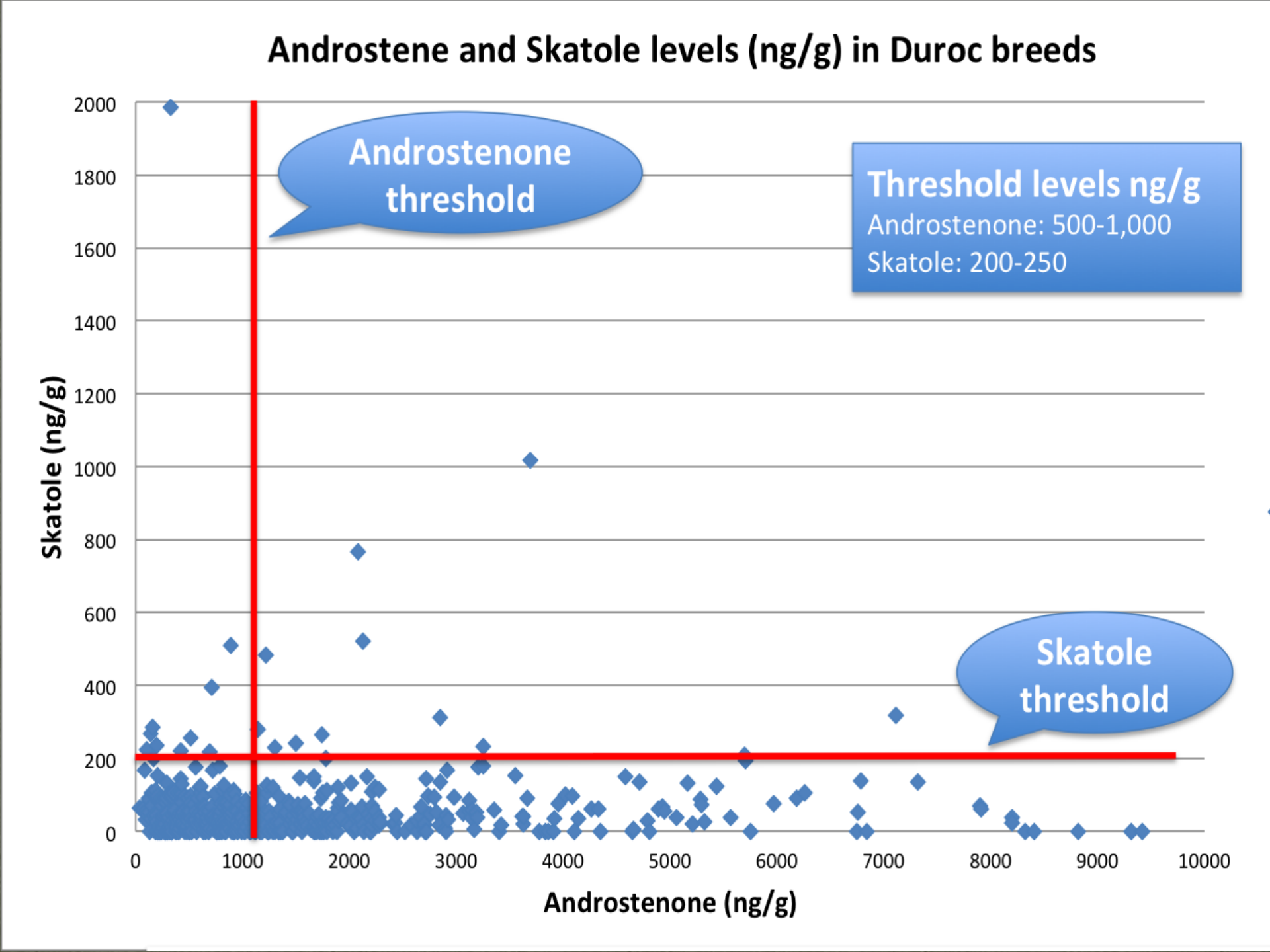
Using genetic markers: Questions to answer

- Is there enough genetic variability in boar taint production?
- Is there enough markers variability?
- Can markers predict boar taint accurately?
  - How effective markers can be used?
- Do markers have any large negative effect on economically important traits?
- Running MEBV (Marker-Assisted EBV)?
  - Use marker relationship in BLUP?
  - Estimate the marker effect in a regression model?
  - Bayesian estimation of breeding values …?

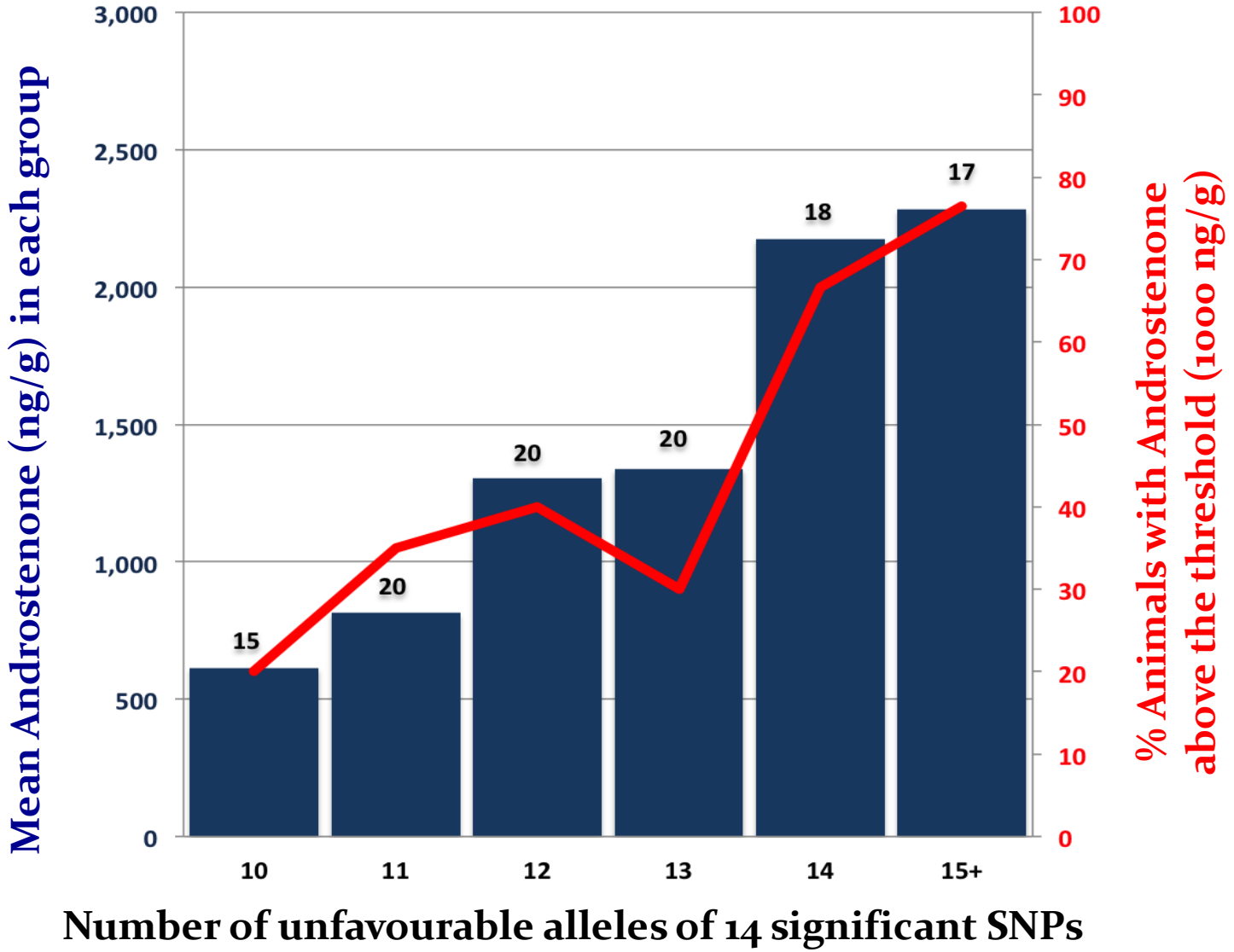
Frequency of 97 genetic markers within major Canadian purebred pigs




Androstene and Skatole levels (ng/g) in maternal breeds
Androstenone threshold
Threshold levels ng/g
Androstenone: 500-1,000
Skatole: 200-250
Skatole threshold
Landrace
Yorkshire
Skatole (ng/g)
Androstenone (ng/g)
0
200
400
600
800
1000
1200
1400
1600
1800
2000
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000


Androstene and Skatole levels (ng/g) in Duroc breeds
Androstenone threshold
Threshold levels ng/g
Androstenone: 500-1,000
Skatole: 200-250
Skatole threshold
Skatole (ng/g)
Androstenone (ng/g)
0
200
400
600
800
1000
1200
1400
1600
1800
2000
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000

Number of unfavourable alleles and levels of Androstenone in Duroc pigs



Prediction of Boar Taint in Major Breeds of Canadian Pigs Using Genetic Markers

M. Jafarikia[1,2], J. Squires[2], F. Schenkel[2], F. Fortin[3]
S. Wyss[1], W. Van Berkel[4], B. Sullivan[1], R. de Wolde[5]

[1]Canadian Centre for Swine Improvement, Ottawa ON; [2]University of Guelph, Guelph ON
[3]Centre de développement du porc du Québec, Québec QC; [4]Western Swine Testing Association, Lacombe AB; [5]Ontario Swine Improvement, Innerkip ON

PROBLEM: Boar taint is an unpleasant odour produced by the accumulation of androstenone and skatole in fat tissues of intact male pigs. Meat produced from intact males containing too much taint can affect the eating quality of pork. Castration is a common practice for preventing boar taint. However, alternative solutions are of interest due mainly to animal welfare concerns. Moreover, in comparison to castrates, raising entire males is also more profitable due to better feed efficiency and leaner carcass (Babol and Squires, 1995).

POTENTIAL SOLUTION: Genetic selection is a potential solution for decreasing boar taint levels in meat from intact males. Technologies are available to scan animals for candidate genes associated with boar taint, which would allow for selection against alleles responsible for increased levels of androstenone or skatole.

OBJECTIVE: To investigate the feasibility of predicting the amount of androstenone and skatole in fat tissues of intact males using SNP markers in candidate genes.

SAMPLING: A total of 3,474 purebred Canadian Duroc, Landrace and Yorkshire boars were sampled and genotyped for 97 SNPs located in 40 candidate genes. SNPs with minor allele frequencies (MAF) less than 0.05 within breed were excluded. Animals weighing less than 90 kg were excluded since they may not have reached sexual maturity. Animals heavier than 150 kg or older than 300 days were also excluded due to potential changes in levels of boar taint compounds over time in older animals. A fat sample was collected from market weight boars at slaughter plants or via biopsies (Baes et al, 2013) for DNA extraction and to measure levels of boar taint compounds.

BOAR TAINT MEASUREMENTS: Fat samples were processed to measure androstenone and skatole levels following to Squires and Lundström (1997) and Lanthier et al. (2007), respectively. The natural logarithms of androstenone and skatole were calculated and used as phenotypes.

VALIDATION: The SAS GLM procedure was used to adjust phenotypes for season, as well as for boar's age and weight at time of sampling. Residuals of the GLM procedure were then used in gebv software (Sargolzaei et al., 2009) to predict the genetic value (VanRaden, 2009) of the boars for androstenone and skatole. The oldest 80% of boars were assigned to a training group and the youngest 20% of boars to a validation group. The correlations of direct genomic values (DGVs) with the adjusted values of androstenone and skatole in the validation group were calculated to validate the predictive ability of marker assisted genetic values for the prediction of boar taint compounds.

Number of boars in training and validation groups

Breed	Androstenone		Skatole	
	Training	Validation	Training	Validation
Duroc	471	115	451	115
Landrace	574	143	544	130
Yorkshire	581	160	554	137

MARKER FREQUENCY: A total number of 61, 80 and 83 SNPs were segregating with a MAF>0.05 in Duroc, Landrace and Yorkshire pigs, respectively.

BOAR TAINT LEVELS: Across all three breeds, about 4% of boars were above the consumer acceptance levels for both measured compounds, 28% were too high for androstenone, but were acceptable for skatole, 6% were too high for skatole, but were acceptable for androstenone and 62% were acceptable for both compounds (table below).

Descriptive statistics on androstenone and skatole levels in Canadian boars

Boar taint compound	Breed		
	Duroc	Landrace	Yorkshire
Androstenone (ng/g)			
Number of animals	588	723	741
Mean	1,467	931	841
Standard deviation	1,567	1,116	875
Range	35-10,419	65-13,748	75-7,747
%Unacceptable	47	28	25
Skatole (ng/g)			
Number of animals	568	680	691
Mean	71	127	94
Standard deviation	129	216	170
Range	0-1,986	0-3,062	0-3,503
%Unacceptable	5	14	10

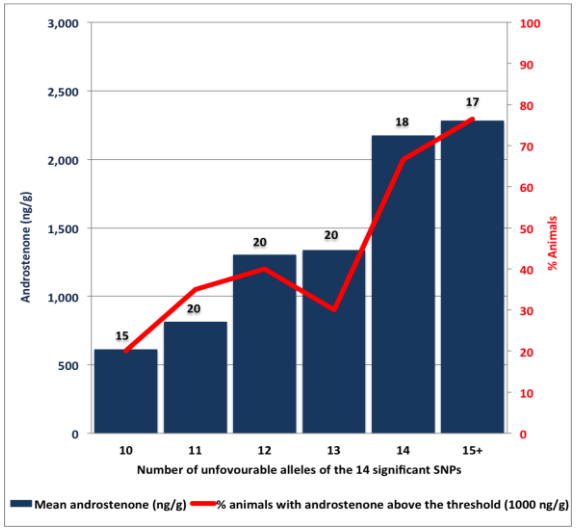
Presented at the CMSA/CMC 2014 Technical Symposium Toronto, Ontario

Acknowledgements: Financial support provided by the Canadian Agricultural Adaptation Program (CAAP), regional swine improvement centres across Canada and participating Canadian breeders.

MARKER ASSISTED GENETIC VALUES ACCURACY: Correlations of the marker assisted genetic values with the adjusted values of androstenone and skatole levels in the validation groups were 0.35 and 0.05 in Duroc, 0.26 and 0.26 in Landrace and 0.40 and -0.05 in Yorkshire, respectively. The low correlations observed for skatole may be a consequence of the lower incidence of skatole values above consumer acceptance thresholds. These results show, however, the potential of the markers as a tool to select breeding animals against high levels of androstenone, the compound most responsible for unacceptable levels of taint in this study.

MARKER FREQUENCY AND LEVELS OF BOAR TAINT: Following the fist step of the analysis, the number of unfavorable SNP alleles associated with androstenone in Duroc was counted on each boar in the validation group. The number of unfavorable alleles were significantly correlated (r= 0.33, p<0.001) with androstenone levels in fat (chart below).

Relationship between number of unfavorable alleles of SNPs and levels of androstenone in Duroc pigs



Potential Application of Genomics to Reduce Boar Taint Levels in Three Canadian Swine Breeds

J. Squires*, M. Jafarikia*,†, F. Schenkel*, S. Wyss†, F. Fortin‡, W. Van Berkel§, R. de Wolde# and B. Sullivan†

*Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, Ontario, Canada, †Canadian Centre for Swine Improvement, Ottawa, Ontario, Canada

‡Centre de développement du porc du Québec, Québec City, Québec, Canada; §Western Swine Testing Association, Lacombe, Alberta, Canada; #Ontario Swine Improvement, Innerkip, Ontario, Canada

INTRODUCTION

- Boar taint is caused by the accumulation of androstenone and skatole in fat tissues and emanates from intact male pig meat.
- Castration of young piglets is a common practice in preventing boar taint.
- Genetic selection to reduce boar taint offers an alternative solution to castration.

OBJECTIVE

- Investigate the possibility of reducing boar taint in fat tissues of swine using genetic markers.

MATERIALS & METHODS

- A total of 976 Duroc, 1128 Landrace and 1193 Yorkshire pigs were genotyped for 97 SNPs located in 40 genes.
- Fat samples on 644 Duroc, 837 Landrace, and 871 Yorkshire boars were collected at the slaughter plant or *via* biopsies (Baes *et al.* 2013. Animal: 714-720).
- Androstenone levels measured using enzyme-linked immunosorbent assay (ELISA) and skatole using high performance liquid chromatography (HPLC).
- Androstenone and skatole levels were skewed and thus log transformed prior to analysis.
- 80% of older boars were assigned to a training set and 20% of youngest pigs to the validation set.
- A two-step analysis was performed:
  1) SAS PROC GLM to adjust phenotypes for season, boar's age and weight.
  2) SAS PROC REG backward elimination to identify significant SNPs.

RESULTS

- Marker frequency: 61, 80 and 83 SNPs had MAF>0.05 for Duroc, Landrace and Yorkshire pigs, respectively.
- Boar taint measures: Duroc boars had the highest androstenone levels whereas higher skatole levels were found in Landrace and Yorkshire breeds.
- Duroc pigs: In the validation set, the number of unfavorable alleles were significantly correlated (r=0.33, p<0.001) with androstenone levels in fat.

Effectiveness of the markers in training sets

Breed	Duroc	Landrace	Yorkshire
Compound	Androstenone	Skatole	Skatole
Significant SNPs	14	23	12
Model R-square	22%	14%	14%

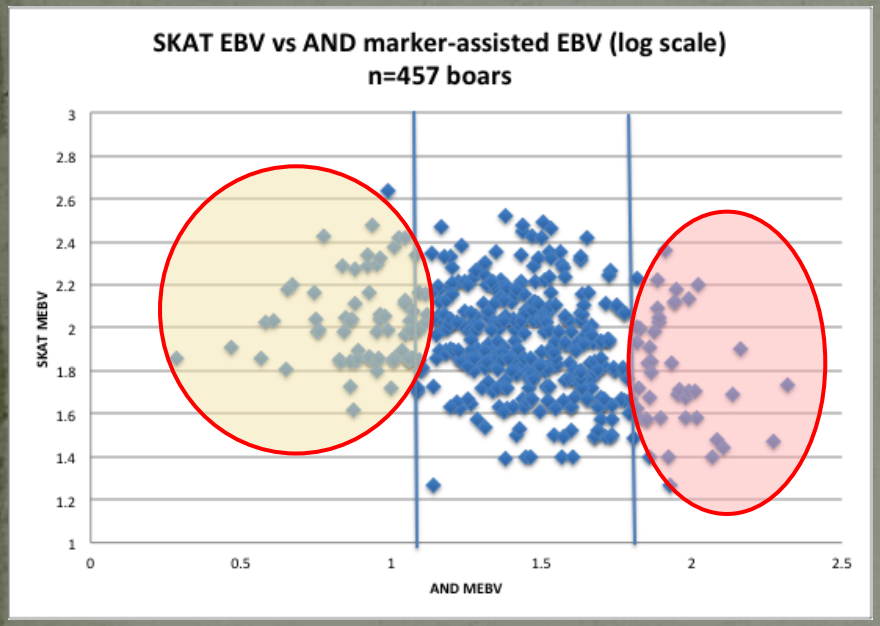
- The percentage of Duroc pigs with androstenone levels above the threshold were 20% and 76% for groups of animals with 10 and 15 or more unfavourable SNP alleles, respectively.
- Landrace pigs: In the validation set, the number of unfavorable alleles was significantly correlated (r=0.23, p<0.01) with skatole levels.
- Yorkshire pigs: No significant correlation between the number of unfavorable alleles and skatole levels were observed in validation set.
  - Only 11% of Yorkshire pigs had high levels of skatole. A larger sample size is recommended.

IMPLICATION & FUTURE WORK

- Markers assisted EBVs (MEBV) for boar taint can be calculated using genetic markers.
- MEBV for boar taint compounds can be included in sire and dam line selection indices.
- Possible negative impacts of markers on production traits should be investigated.
- Efficiency of markers must be validated in commercial pig populations.

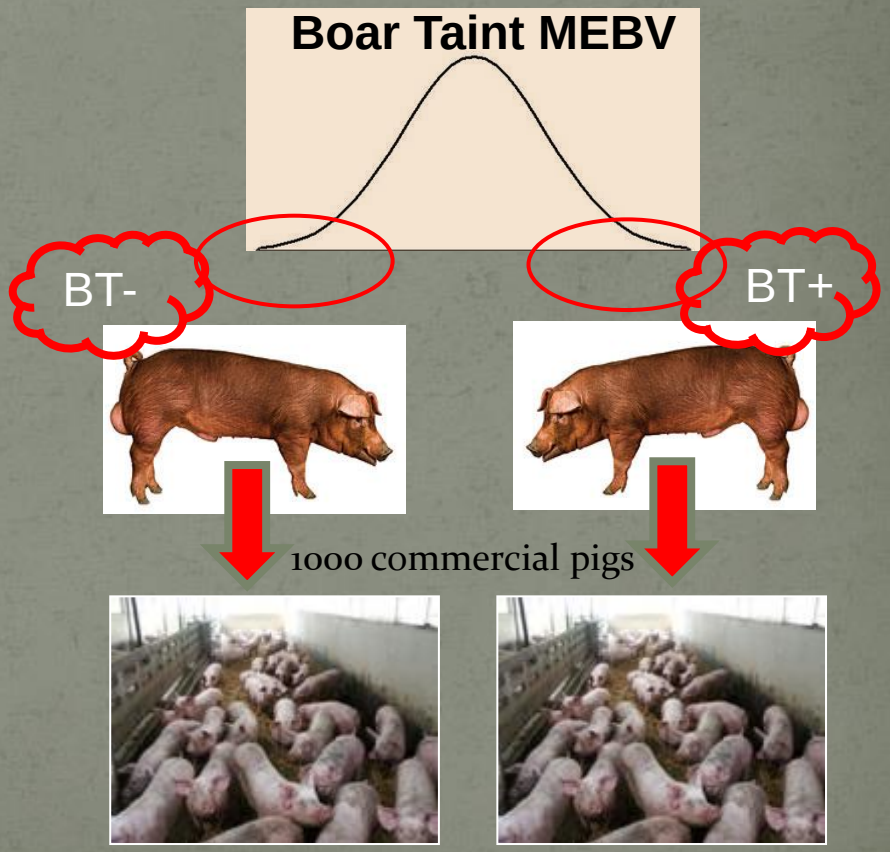
Acknowledgements: Investment in this project was provided by Agriculture and Agri-Food Canada through the Canadian Agricultural Adaptation Program (CAAP). In Ontario, this program was delivered by the Agricultural Adaptation Council. Financial support was also provided by regional swine improvement centres across Canada and participating Canadian breeders.

Need for validation Commercial trials



Bottom 15%

Top 15%

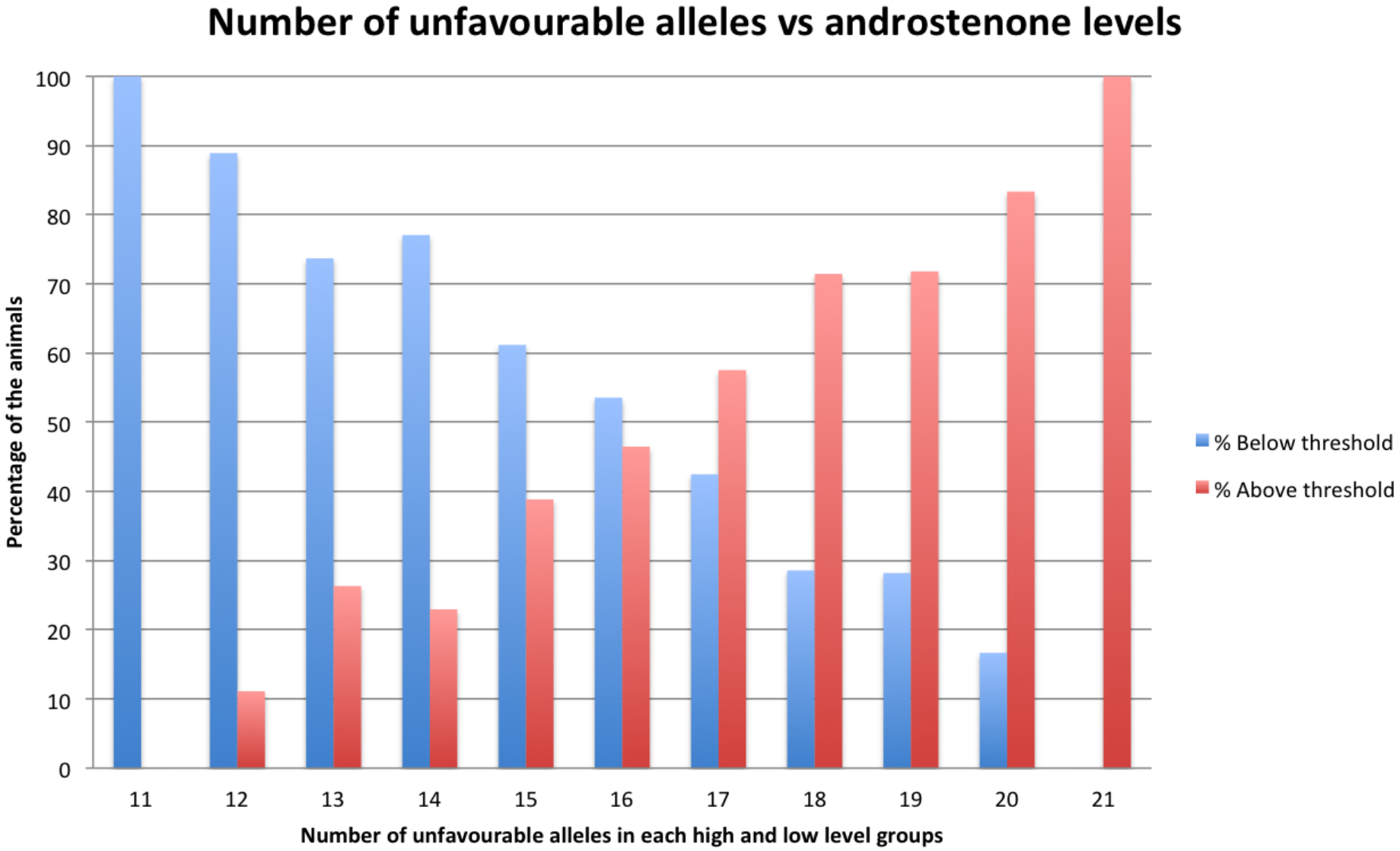


Males, females, castrates, immunocastrates

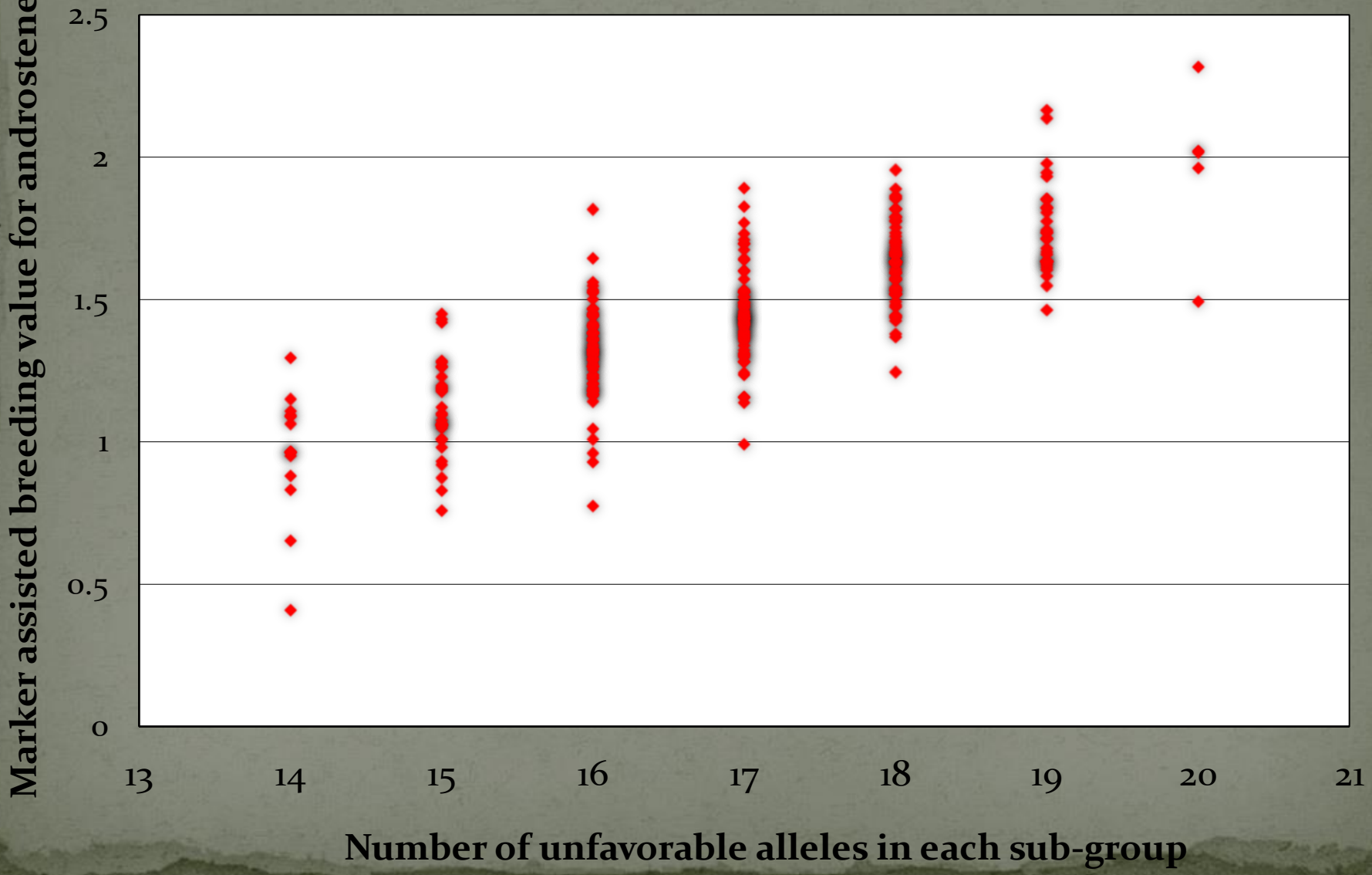
Analysis & Model of Prediction

- Boars 90-150kg and less than 300 days old
- Out of ~100 SNPs, 60 had MAF > 0.05
- Estimation Group: 627 boars
  - Log-transforming of the measurements
  - Adjusting for season, age and weight: SAS PROC GLM
  - Association test: SAS PROC REG Backward Elimination
  - Verifying the results
    - Using solutions to predict boar taint
    - Compare predicted values with real measurements
- Prediction Group: 452 boars to be evaluated as candidate sire
  - Marker predicted values from estimation group

Estimation Group Solutions



Prediction Group Solutions



Status of the commercial trial

- Target is 1,000 progeny tested in three groups
- Includes a gilt, castrate, immunocastrate and intact male from each litter (25% of each sex)
- First group just completed test, second group will complete test this fall and third group in spring 2016

Status of the commercial trial

- From the first group, expected difference in progeny of BT+ and BT- sires is:

  (1.82-0.94)/2=0.44 (log scale)

- Trial results will test accuracy of this prediction
- A taste panel will evaluate pork from each sex
- Also looking at methods to screen carcasses in commercial packing plants
- Looking forward to present results at 2016 EAAP

Acknowledgements

Canadian Centre for Swine Improvement and its Regional Centres

Breeders and other private organizations

Researchers

Financial support

Agriculture and Agri-Food Canada
AgriInnovation Program

Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec