# Genetic analysis of heat tolerance for production and health traits in US Holstein cows

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# Introduction

- ✓ Cows are most efficient at 40 to 70 $^{0}$F
- ✓ Daily temperature above 85 $^{0}$F leads to heat stress in dairy cows
- ✓ High temperature and high humidity has profound effects on dairy cows
  - reduces DMI
  - decreases milk yield
  - depresses fertility
  - increases incidence of health disorders

# Introduction

- ✓ Temperature-humidity index (THI) determines heat stress potential in dairy cattle

- ✓ Derived by an equation from RH and air temperature for a particular day

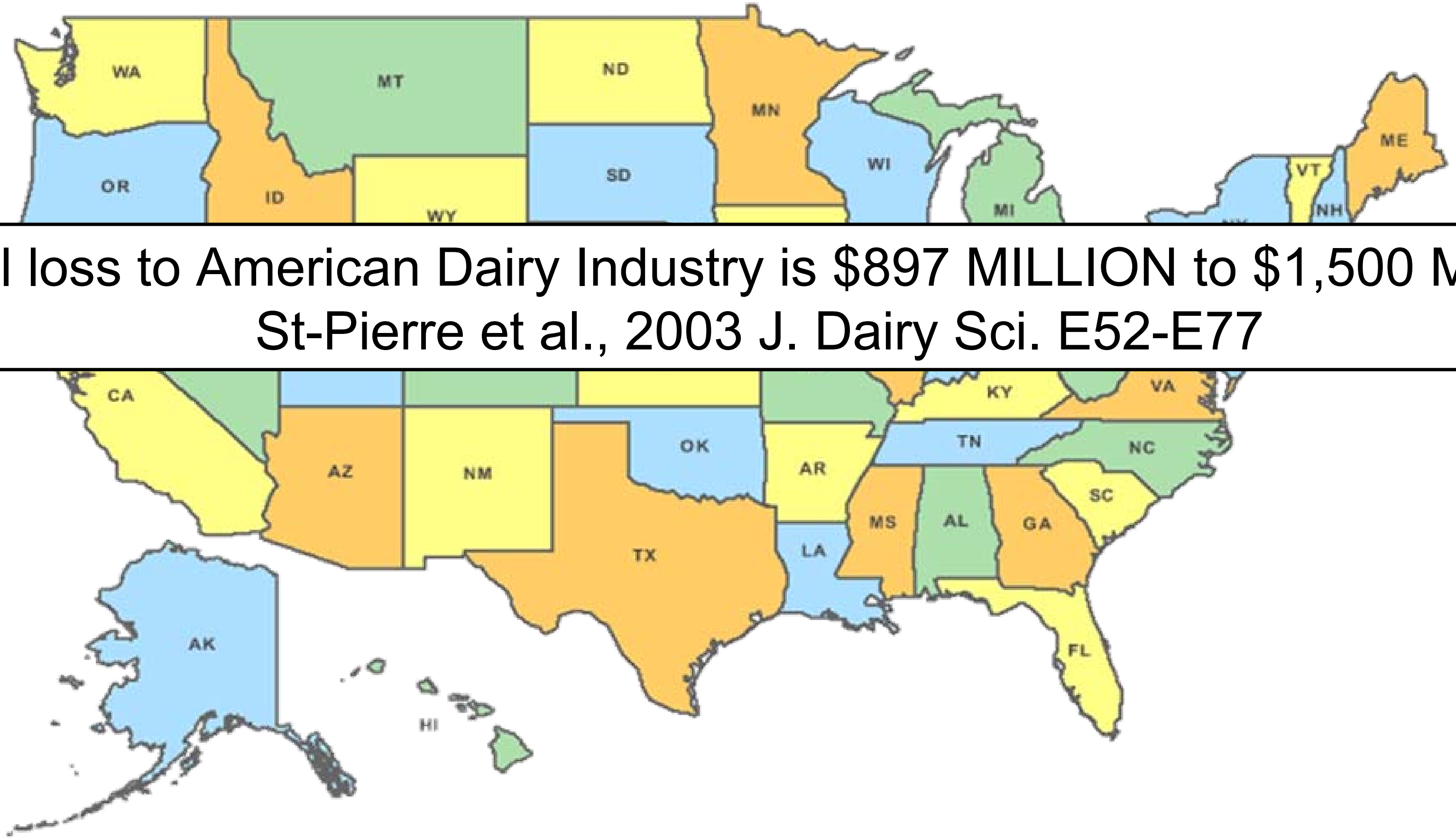
THI (t, rh) = $(1.8 \times t\,^{o}c + 32) - (0.55 - 0.0055 \times rh) \times (1.8 \times t\,^{o}c - 26)$

- ✓ Minimum heat stress threshold of 68 for high producing dairy cows ( ≥ 35 kg/day)

| Themperature | | % Relative Humidity | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| °F | °C | 0 | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 60 | 65 | 70 | 75 | 80 | 85 | 90 |
| 72 | 22.0 | 64 | 65 | 65 | 65 | 66 | 66 | 67 | 67 | 67 | 68 | 68 | 69 | 69 | 69 | 70 | 70 | 70 | 71 | 71 |
| 73 | 23.0 | 65 | 65 | 66 | 66 | 66 | 67 | 67 | [illegible] | 68 | 68 | 69 | 69 | 70 | 70 | 71 | 71 | 71 | 72 | 72 |
| 74 | 23.5 | 65 | 66 | 66 | 67 | 67 | 67 | 68 | [illegible] | 69 | 69 | 70 | 70 | 70 | 71 | 71 | 72 | 72 | 73 | 73 |
| 75 | 24.0 | 66 | 66 | 67 | 67 | 68 | 68 | [illegible] | 69 | 69 | 70 | 70 | 71 | 71 | 72 | 72 | 73 | 73 | 74 | 74 |
| 76 | 24.5 | 66 | 67 | 67 | 68 | 68 | 69 | [illegible] | 70 | 70 | 71 | 71 | 72 | 72 | 73 | 73 | 74 | 74 | 75 | 75 |
| 77 | 25.0 | 67 | 67 | 68 | 68 | 69 | [illegible] | 70 | 70 | 71 | 71 | 72 | 72 | 73 | 73 | 74 | 74 | 75 | 75 | 76 |
| 78 | 25.5 | 67 | 68 | 68 | 69 | [illegible] | 70 | 70 | 71 | 71 | 72 | 73 | [illegible] | 74 | 74 | 75 | 75 | 76 | 76 | 77 |
| 79 | 26.0 | 67 | 68 | 69 | 69 | [illegible] | 70 | 71 | 71 | 72 | 73 | 73 | [illegible] | 74 | 75 | 76 | 76 | 77 | 77 | 78 |
| 80 | 26.5 | 68 | 69 | 69 | [illegible] | 70 | 71 | 72 | 72 | 73 | 73 | [illegible] | 75 | 75 | 76 | 76 | 77 | 78 | 78 | 79 |
| 81 | 27.0 | 68 | 69 | [illegible] | [illegible] | 71 | 72 | 72 | 73 | 73 | 74 | [illegible] | 75 | 76 | 77 | 77 | 78 | 78 | 79 | 80 |
| 82 | 28.0 | 69 | [illegible] | [illegible] | 71 | 71 | 72 | 73 | 73 | 74 | [illegible] | 75 | 76 | 77 | 77 | 78 | 79 | 79 | 80 | 81 |
| 83 | 28.5 | 69 | 70 | 71 | 71 | 72 | 73 | 73 | 74 | [illegible] | 75 | 76 | 77 | 78 | 78 | 79 | 80 | 80 | 81 | 82 |
| 84 | 29.0 | 70 | 70 | 71 | 72 | 73 | 73 | 74 | 75 | [illegible] | 76 | 77 | 78 | 78 | 79 | 80 | 80 | 81 | 82 | 83 |
| 85 | 29.5 | 70 | 71 | 72 | 72 | 73 | 74 | 75 | [illegible] | 76 | 77 | 78 | 78 | 79 | 80 | 81 | [illegible] | 82 | 83 | 84 |
| 86 | 30.0 | 71 | 71 | 72 | 73 | 74 | 74 | 75 | [illegible] | 77 | 78 | 78 | 79 | 80 | 81 | 81 | [illegible] | 83 | 84 | 84 |
| 87 | 30.5 | 71 | 72 | 73 | 73 | 74 | 75 | [illegible] | 77 | 77 | 78 | 79 | 80 | 81 | 81 | [illegible] | 83 | 84 | 85 | 85 |
| 88 | 31.0 | 72 | 72 | 73 | 74 | 75 | [illegible] | 76 | 77 | 78 | 79 | 80 | 81 | 81 | 82 | [illegible] | 84 | 85 | 86 | 86 |
| 89 | 31.5 | 72 | 73 | 74 | 75 | [illegible] | [illegible] | 77 | 78 | 79 | 80 | 80 | 81 | 82 | [illegible] | 84 | 85 | 86 | 86 | 87 |
| 90 | 32.0 | 72 | 73 | 74 | 75 | [illegible] | 77 | 78 | 79 | 79 | 80 | 81 | 82 | [illegible] | [illegible] | 85 | 86 | 86 | 87 | 88 |
| 91 | 33.0 | 73 | 74 | 75 | 76 | [illegible] | 77 | 78 | 79 | 80 | 81 | 82 | 83 | [illegible] | 85 | 86 | 86 | 87 | 88 | 89 |
| 92 | 33.5 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | [illegible] | 85 | 85 | 86 | 87 | 88 | 89 | 90 |
| 93 | 34.0 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 80 | 81 | 82 | 83 | [illegible] | 85 | 86 | 87 | 88 | 89 | 90 | 91 |
| 94 | 34.5 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | [illegible] | 86 | 86 | 87 | 88 | 89 | 90 | 91 | 92 |
| 95 | 35.0 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | [illegible] | [illegible] | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 |
| 96 | 35.5 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | [illegible] | [illegible] | 86 | 87 | 88 | 89 | 90 | 91 | [illegible] | 93 | 94 |
| 97 | 36.0 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | [illegible] | 85 | 86 | 87 | 88 | 89 | 91 | 92 | [illegible] | 94 | 95 |
| 98 | 36.5 | 76 | 77 | 78 | 80 | 80 | 82 | 83 | [illegible] | [illegible] | 86 | 87 | 88 | 89 | 90 | 91 | [illegible] | 93 | 94 | 95 |
| 99 | 37.0 | 76 | 78 | 79 | 80 | 81 | 82 | 83 | [illegible] | 85 | 87 | 88 | 89 | 90 | 91 | 92 | [illegible] | 94 | 95 | 96 |
| 100 | 38.0 | 77 | 78 | 79 | 81 | 82 | 83 | 84 | [illegible] | 86 | 87 | 88 | 90 | 91 | 92 | [illegible] | 94 | 95 | 96 | 98 |
| 101 | 38.5 | 77 | 79 | 80 | 81 | 82 | 83 | 84 | 86 | 87 | 88 | 89 | 90 | 92 | [illegible] | [illegible] | 95 | 96 | 98 | 99 |
| 102 | 39.0 | 78 | 79 | 80 | 82 | 83 | 84 | 85 | 86 | 87 | 89 | 90 | 91 | 92 | [illegible] | 95 | 96 | 97 | 98 | 100 |
| 103 | 39.5 | 78 | 79 | 81 | 82 | 83 | 84 | 86 | 87 | 88 | 89 | 91 | 92 | [illegible] | 94 | 96 | 97 | 98 | 99 | 101 |
| 104 | 40.0 | 79 | 80 | 81 | 83 | 84 | 85 | 86 | 88 | 89 | 90 | 91 | 93 | [illegible] | 95 | 96 | 98 | 99 | 100 | 101 |
| 105 | 40.5 | 79 | 80 | 82 | 83 | 84 | 86 | 87 | 88 | 89 | 91 | 92 | 93 | 95 | 96 | 97 | 99 | 100 | 101 | 102 |
| 106 | 41.0 | 80 | 81 | 82 | 84 | 85 | 87 | 88 | 89 | 90 | 91 | 93 | 94 | 95 | 97 | 98 | 99 | 101 | 102 | 103 |
| 107 | 41.5 | 80 | 81 | 83 | 84 | 85 | 87 | 88 | 89 | 91 | 92 | 94 | 95 | 96 | 98 | 99 | 100 | 102 | 103 | 104 |

Stress threshold
Mild moderate stress
Moderate severe stress
Moderate stress
Severe stress

# Heat Stress in the United States




Annual loss to American Dairy Industry is $897 MILLION to $1,500 MILLION !
St-Pierre et al., 2003 J. Dairy Sci. E52-E77

# Heat Stress Management

- ✓ Different cooling and nutritional strategies are used to alleviate effects of heat stress
- ✓ However, production continues to decline during summer
- ✓ Identifying heat tolerant cows and understanding biological mechanism of thermotolerance is critical for developing novel approach
- ✓ Breeding for thermotolerance is permanent, cumulative and cost effective approach

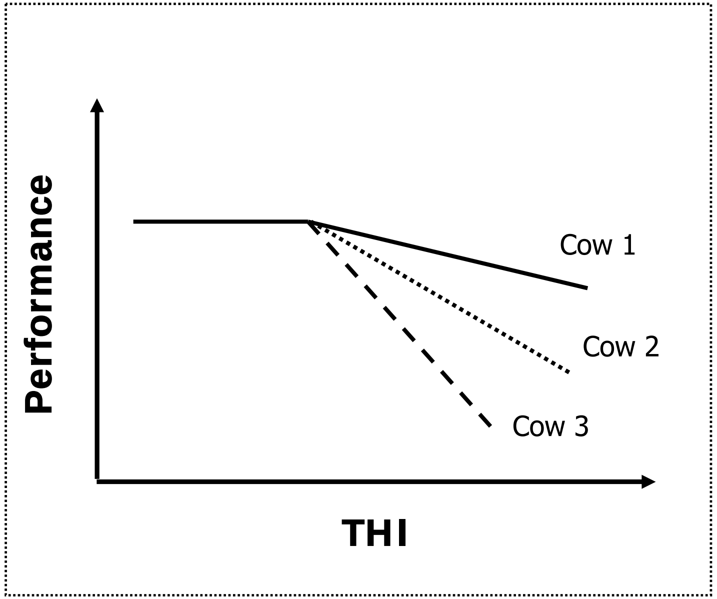



# Objectives

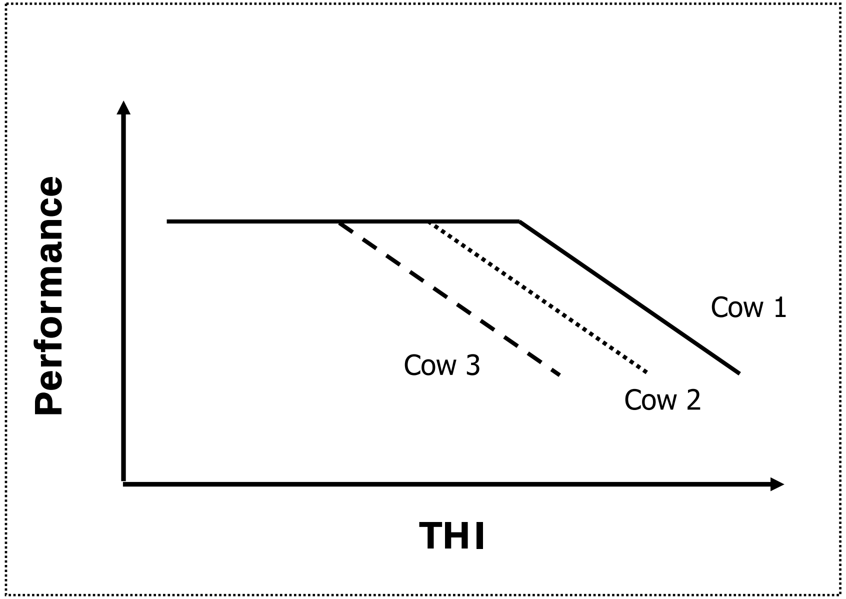
- ❑ Estimate genetic components of milk yield (MY) and somatic cell score (SCS) across lactations considering heat stress
- ❑ Identify and characterize genomic regions, and preferably individual genes and pathways responsible for heat tolerance in MY and SCS

# Modeling Heat Stress

Aim: identify animals that under heat stress conditions show either none or low rates of performance decline or delayed onset of performance decline




**different rates of performance decline**




**differences on the onset of decline**

*Source: I. Misztall and collaborators (2000)*

# Materials and Methods

- ✓ Phenotype

| | Cows, n | Records, n | Test days, n | Min, TD records | Mean ± SD |
|---|---|---|---|---|---|
| **MILK** | 17,522 | 254,215 | 278 | 8 | 35.87 ±8.26 |
| **SCS** | 18,975 | 355,546 | 293 | 5 | 1.96 ±1.81 |

- ✓ Genotype: **6k** animals (1592 sires + 4770 cows) (60k SNP across the genome)
- ✓ Pedigree : **31k** animals ( A 5-generation pedigree from CDCB)
- ✓ Merge Test day records with weather records: mean daily THI of 3 days prior the test day (Bohmanova et al. (2007) )

# Statistical Model

## Model: Multi-trait Repeatability Test Day Model

$$y_{klmn} = \mathrm{HTD}_{kl} + \mathrm{DIM}_m + \boldsymbol{a}_{(\boldsymbol{general})\boldsymbol{n}} + \boldsymbol{v}_{\boldsymbol{n}}\,[\phi\,(\boldsymbol{THI})\,] + \boldsymbol{P}_{(\boldsymbol{general})\boldsymbol{n}} + \boldsymbol{q}_{\boldsymbol{n}}\,[\phi\,(\boldsymbol{THI})\,] + \mathrm{e}_{klmn}$$

## Statistical Analysis

- ✓ A function of THI (ф (thi)) was created to estimate the effects of heat stress

$$\Phi\,(\mathrm{THI}) = \begin{cases} 0 \\ THI - THI_{Threshold} \end{cases}$$

- ✓ Variance components and genetic parameters were estimated in Bayesian framework using GIBBS2F90 ( Blupf90 family of programs)

- ✓ Heritability at heat stress level f(i) was calculated as:

$$h^2 = \frac{\sigma_a^2 + f(i)^2\sigma_v^2 + 2\mathrm{f(i)}\sigma_{av}}{\sigma_a^2 + f(i)^2\sigma_v^2 + 2\mathrm{f(i)}\sigma_{av} + \sigma_p^2 + f(i)^2\sigma_q^2 + 2\mathrm{f(i)}\sigma_{pq} + \sigma_e^2}$$

# GWAS and Gene set analysis

**ssGWAS:** Genetic variance explained by 2.0Mb window of adjacent SNPs

**Gene set analysis**

1. assignment of **SNPs** to **genes**

criteria: SNP **within the gene** or at most **15kb** either **upstream/downstream** the gene

2. assignment of **genes** to **functional categories**

- databases: Gene Ontology, Medical Subject Headings (MeSH)

3. association analysis between **each functional term** and the **phenotype of interest based on** Fisher's exact test

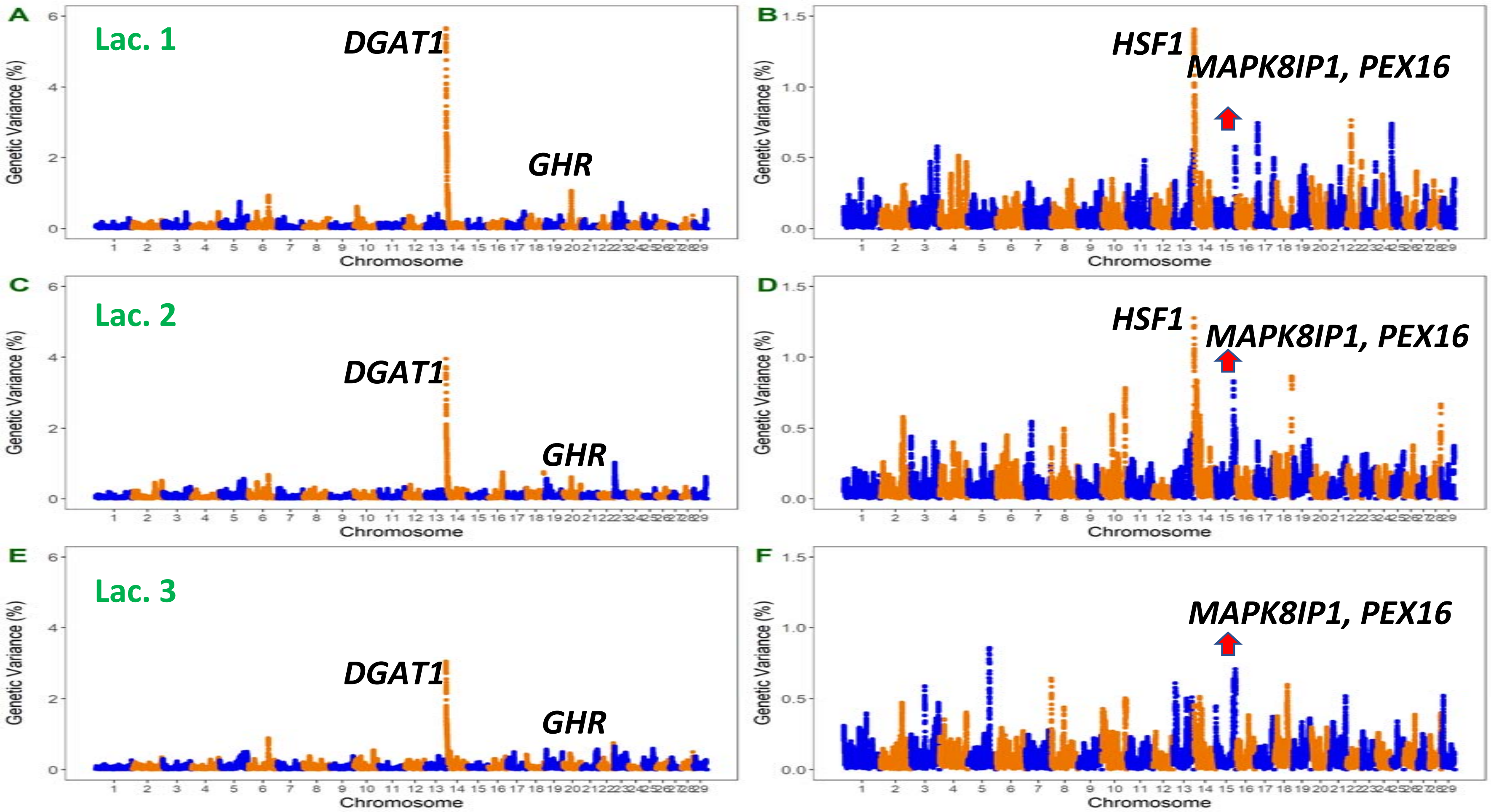
# Results and Discussion

- ✓ Variance component estimates
- ✓ GWAS
- ✓ Gene set analysis

# MILK YIELD (MY)

| Parameters | Parity1 | Parity2 | Parity3 |
|---|---|---|---|
| $\sigma^2_a$ | 9.26 | 10.03 | 10.55 |
| $100\sigma^2_v$ | 0.94 | 1.56 | 1.62 |
| $10\sigma_{a.v}$ | -1.21 | -1.17 | -2.31 |
| $h^2_{f(10)}$ | 0.32 | 0.24 | 0.17 |
| $r^G_{(a.v)}$ | -0.41 | -0.30 | -0.55 |
| Cor-ht(par1,parj) | | 0.78 | 0.65 |
| Cor-ht(par2,parj) | | | 0.61 |
| Cor-gen(par1,parj) | | 0.82 | 0.85 |
| Cor-gen(par2,parj) | | | 0.92 |

# MILK YIELD (MY)



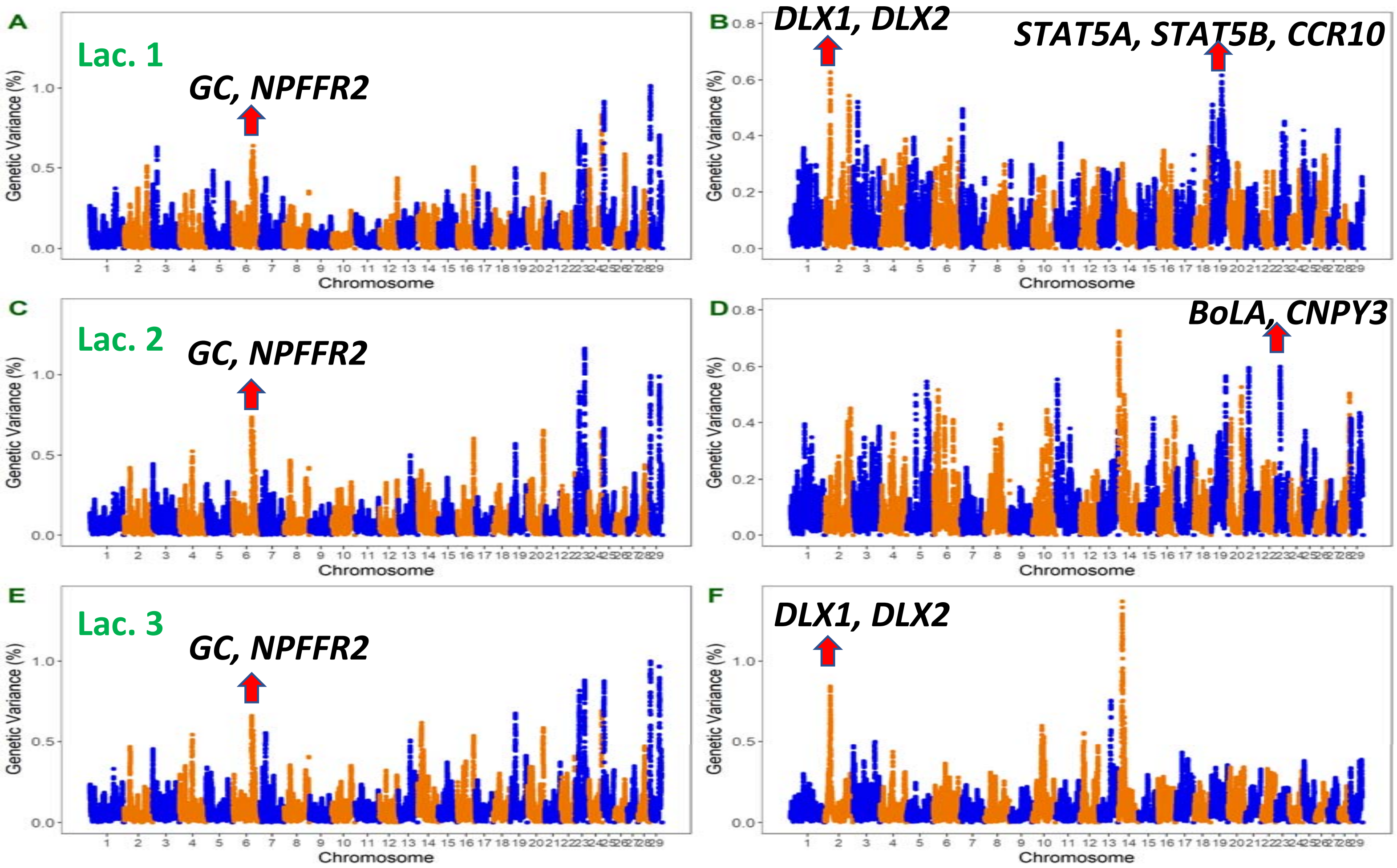
# MILK YIELD (MY)

| GO ID | GO TERM | No. of Genes | No. of Sig. Genes | P_value |
|---|---|---|---|---|
| *GO:0034605* | ***cellular response to heat*** | 15 | 3 | 0.003 |
| GO:0009266 | *response to temperature stimulus* | 33 | 3 | 0.027 |
| GO:0080135 | *cellular response to stress* | 96 | 5 | 0.043 |
| *GO:2001020* | ***response to DNA damage stimulus*** | 35 | 3 | 0.032 |
| *GO:0050848* | *calcium-mediated signaling* | 15 | 3 | 0.003 |
| *GO:0048016* | *Inositol phosphate mediated signaling* | 14 | 2 | 0.030 |
| *GO:0009065* | *glutamine family amino acid catabolic process* | 9 | 2 | 0.013 |
| **MeSH ID** | **MeSH Term** | | | |
| *D018869* | ***Heat-Shock Response*** | 9 | 2 | 0.015 |
| D050886 | ***HSP20 Heat-Shock Proteins*** | 2 | 1 | 0.004 |
| D005982 | *Glutathione Transferase* | 50 | 4 | 0.002 |
| D054732 | *Calcium-Calmodulin-Dependent Protein Kinase Type 2* | 13 | 2 | 0.002 |

# SOMATIC CELL SCORE (SCS)

| Parameters | Parity1 | Parity2 | Parity3 |
| --- | --- | --- | --- |
| $\sigma^2_a$ | 0.27 | 0.28 | 0.38 |
| $100\sigma^2_v$ | 0.042 | 0.049 | 0.091 |
| $10\sigma_{a.v}$ | 0.026 | 0.011 | 0.081 |
| $h^2_{f(10)}$ | 0.11 | 0.10 | 0.15 |
| $r^G_{(a.v)}$ | 0.24 | 0.10 | 0.43 |
| Cor-ht(par1,parj) | | 0.18 | 0.68 |
| Cor-ht(par2,parj) | | | 0.33 |
| Cor-gen(par1,parj) | | 0.77 | 0.79 |
| Cor-gen(par2,parj) | | | 0.90 |

# SOMATIC CELL SCORE (SCS)



# SOMATIC CELL SCORE (SCS)

| GO ID | GO TERM | No. of Genes | No. of Sig. Genes | P_value |
|---|---|---|---|---|
| GO:0061077 | ***Chaperone-mediated protein folding*** | 26 | 3 | 0.016 |
| GO:0006260 | ***DNA replication*** | 57 | 4 | 0.031 |
| GO:0050671 | *Positive regulation of lymphocyte proliferation* | 27 | 4 | 0.002 |
| GO:0006909 | *phagocytosis* | 31 | 4 | 0.003 |
| GO:0050727 | ***Regulation of inflammatory response*** | 56 | 4 | 0.029 |
| GO:0042742 | *defense response to bacterium* | 41 | 4 | 0.007 |
| **MeSH ID** | **MeSH Term** | | | |
| D008285 | ***Major Histocompatibility Complex*** | 14 | 2 | 0.036 |
| D007113 | *Immunity Innate* | 35 | 4 | 0.004 |
| D007375 | ***Interleukin-1*** | 29 | 3 | 0.012 |

# Conclusions

- Reinforces the idea that relationship of production with thermotolerance is antagonistic
- Continued selection for general merit will result in greater susceptibility to heat stress
- GWAS and Gene set analysis: list of putative candidate regions and genes with known roles in general merit and heat tolerance identified

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COUNCIL ON DAIRY CATTLE BREEDING

**Thank you**
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