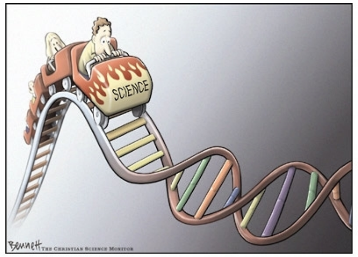
Breeding Approaches to Improve Robustness and Resilience in Dairy Cows

Sarah Zakrzewski de Saurith, Katharina May, Sven König

Institute of Animal Breeding and Genetics, Justus-Liebig-University, Gießen, Germany



Robustness

What makes a cow “robust”?

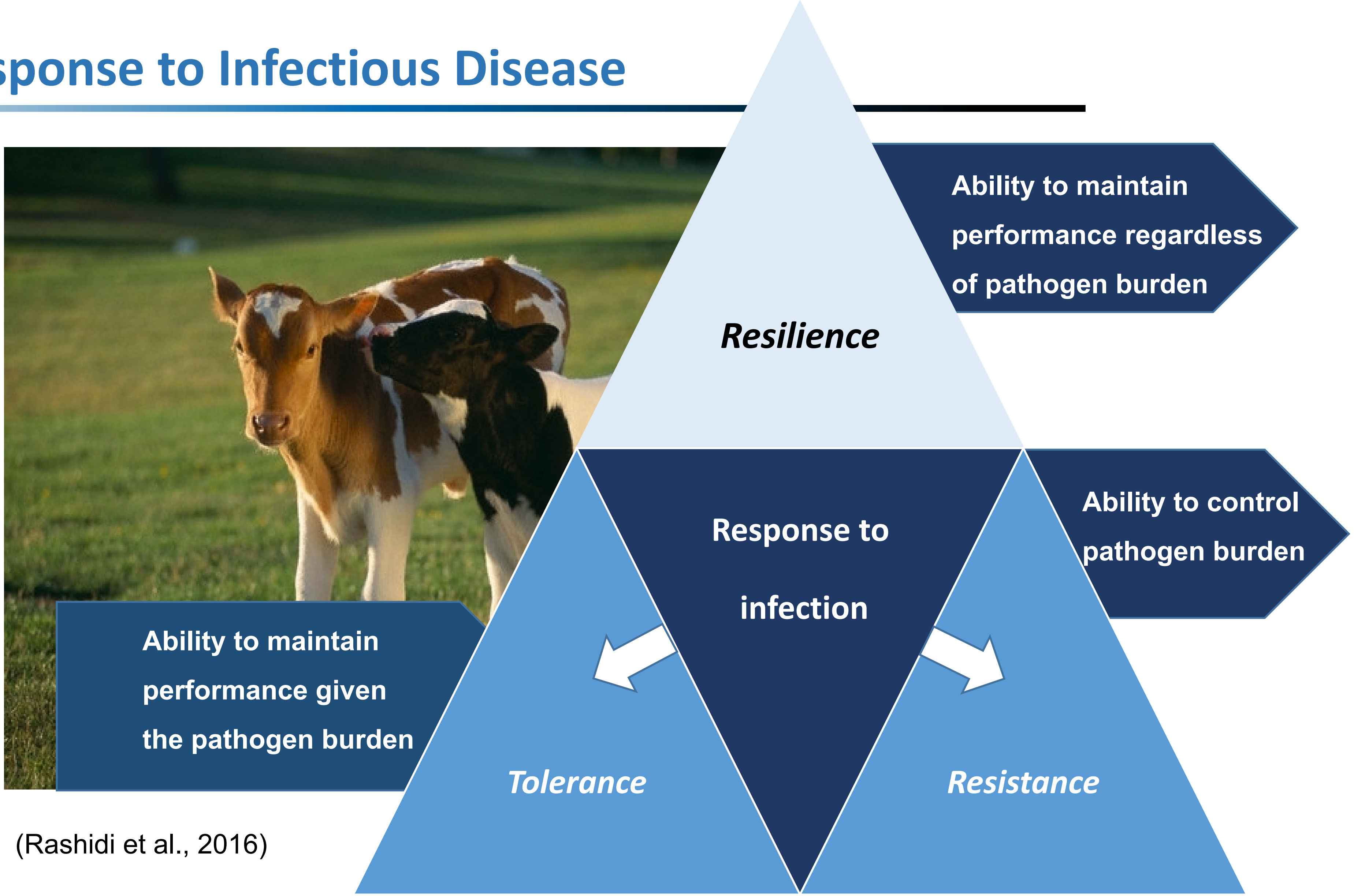
Definition: Animals that maintain a relatively constant level of productivity, health, and fertility, independent from environmental alterations (De Jong and Bijma, 2002)

If we define robustness as such, we need to examine:

- Cow health concerns (parasitic infection)
- Heat stress

A robust cow one that is **resilient** to these factors.

Host Response to Infectious Disease



Resilience

Ability to maintain performance regardless of pathogen burden

Response to infection

Tolerance

Ability to maintain performance given the pathogen burden

Resistance

Ability to control pathogen burden

(Rashidi et al., 2016)

Cattle Health Concerns- Parasitic Infection

Our department at the university has been working extensively to examine parasitic load of cattle, specifically endoparasitic infections.

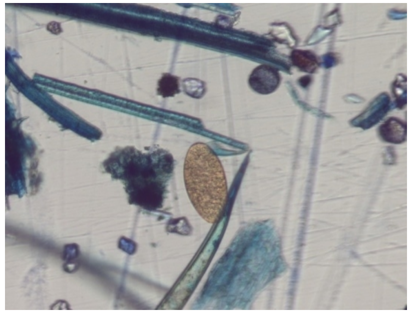
Collection of fecal samples and analysis

Gastrointestinal nematodes
(egg counts)

FEC-GIN

Bovine lungworms
(larvae counts)

FLC-DV



Liver flukes
(egg counts)

FEC-FLU

PASTURE GENETIC STUDY: Genetic Line Comparisons

German Holstein cow

HF-GHm

x GH „milk sires“

Selected for milk yield

HF-GHp

x GH “pasture sires“

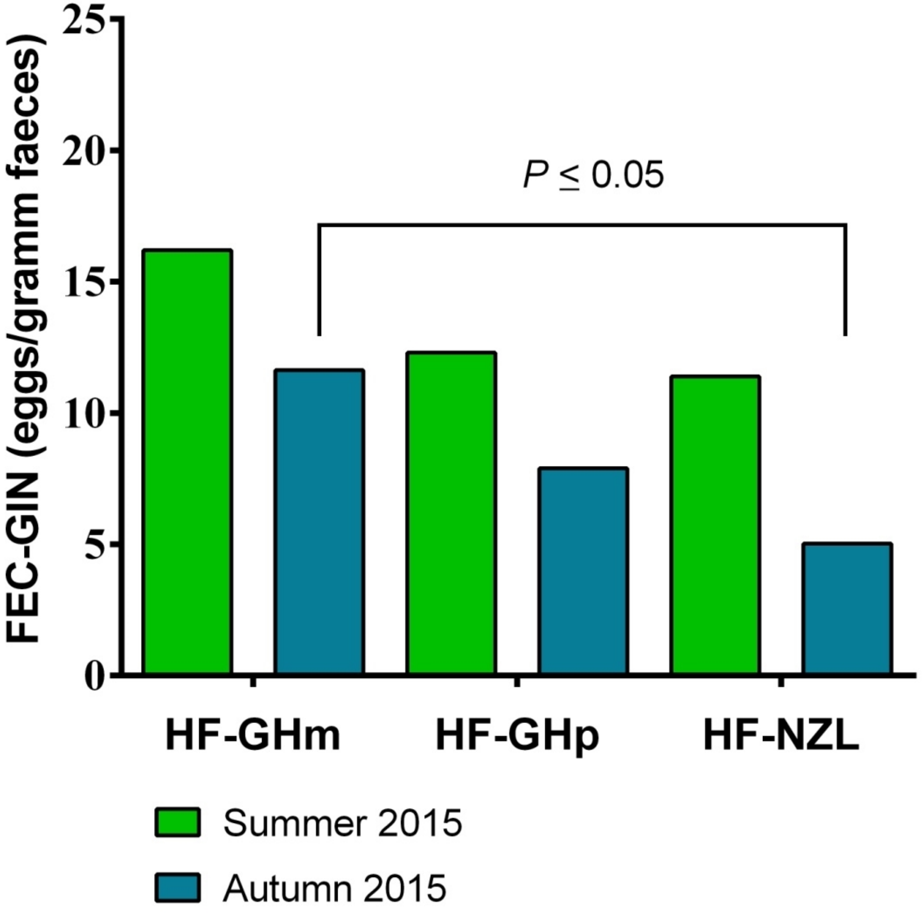
Selected for pasture traits

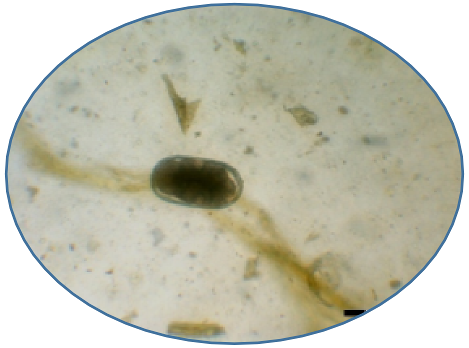
HF-NZL

x HF-NZL sires

Selected for NZ genetics

Endoparasite Resistance: Genetic Line Comparisons





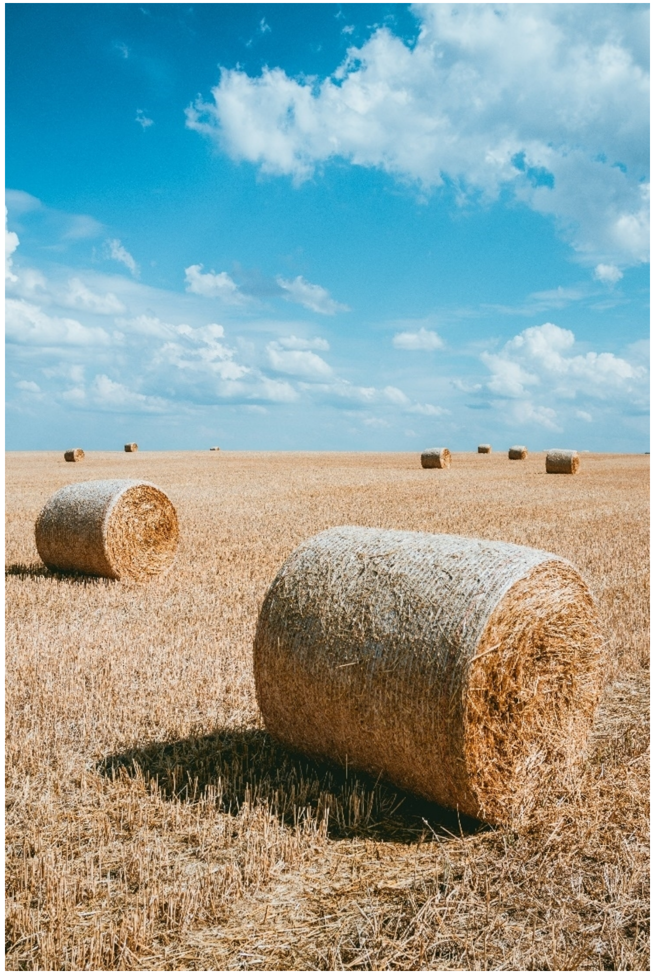
- **Lower egg counts** for gastrointestinal nematodes **in HF-NZL cows**

- **Lower egg counts for pasture adapted lines (HF-GHp)** in comparison to HF-GHm
- Resistance to endoparasites is of genetic nature

Heat Stress

Test the environmental sensitivity of our results

- Genotype- environment interactions (**G x E**)
- Heat stress as the environmental factor

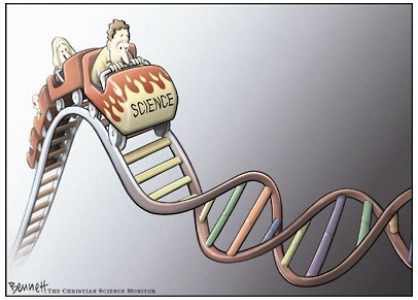


Modern G x E – Studies: What Do We Need?

A: Novel health traits / detailed phenotypes

„In the era of genomics, phenotype is king!“
(M. Coffey, 2010, WCGALP, Leipzig, Germany)

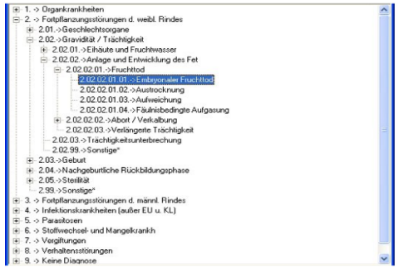
B: Novel and dense genome data



C: Novel continuous environmental gradients

Data Source in Germany

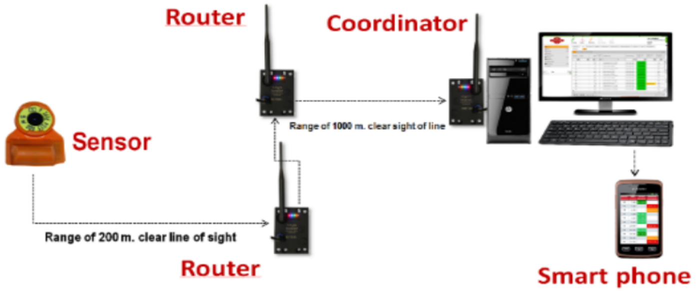
- **Cow data:**
  - 22.212 Holstein cows with 38.107 lactations
  - cows are kept in 25 contract herds
  - dense genotype (50K or 777K) or genome sequence data
  - ongoing process since 2016 (implementation funded by the German Ministry of Education and Research (BMBF))
  - novel functional health and health indicator traits



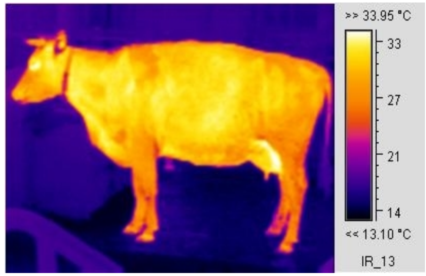
- 1. -> Organkrankheiten
- 2. -> Fortpflanzungsstörungen d. weibl. Rindes
  - 2.01.->Geschlechtsorgane
  - 2.02.->Gravidität / Trächtigkeit
    - 2.02.01.->Eihäute und Fruchtwasser
    - 2.02.02.->Anlage und Entwicklung des Fet
      - 2.02.02.01.->Fruchttod
        - 2.02.02.01.01.->Embryonaler Fruchttod
        - 2.02.02.01.02.->Austrocknung
        - 2.02.02.01.03.->Aufweichung
        - 2.02.02.01.04.->Fäulnisbedingte Aufgasung
      - 2.02.02.02.->Abort / Verkalbung
      - 2.02.02.03.->Verlängerte Trächtigkeit
    - 2.02.03.->Trächtigkeitsunterbrechung
    - 2.02.99.->Sonstige*
  - 2.03.->Geburt
  - 2.04.->Nachgeburtliche Rückbildungsphase
  - 2.05.->Sterilität
  - 2.99.->Sonstige*
- 3. -> Fortpflanzungsstörungen d. männl. Rindes
- 4. -> Infektionskrankheiten (außer EU u. KL)
- 5. -> Parasitosen
- 6. -> Stoffwechsel- und Mangelkrankh
- 7. -> Vergiftungen
- 8. -> Verhaltensstörungen
- 9. -> Keine Diagnose

Health diagnoses

Health Biomarkers



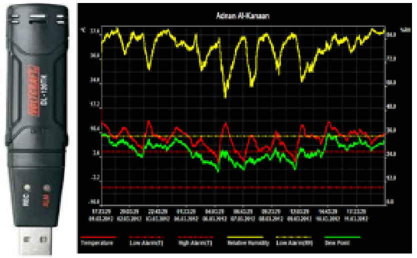
Health indicators



Physiological traits

Microbiota and endoparasites

- **Climate data:**
  - measurements of THI (temperature humidity index) in the barn via Voltcraft (DL-120 TH) in intervals of 30 minutes

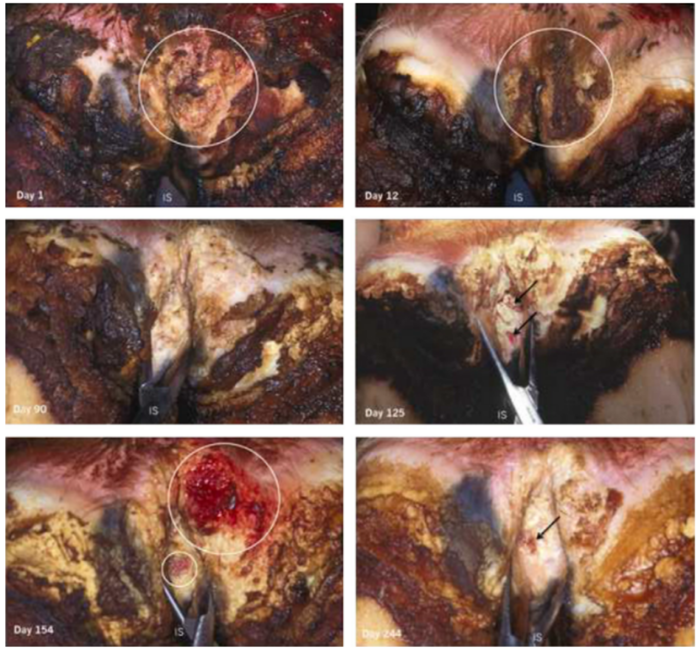


Accurate Disease Definition ==> Higher Heritabilities

Schöpke et al., J. Dairy Sci., 2015

Application of a more detailed scoring system for digital dermatitis (DD)

Trait	Heritability
DD Binary trait 1	0.19 ± 0.11
M-stage (M2 lesion)	0.52 ± 0.17
Change of M-stage	0.42 ± 0.15



(Figure from Berry et al., 2012)

M-score system

6 stages of DD

Genetic Statistical Modelling Approach

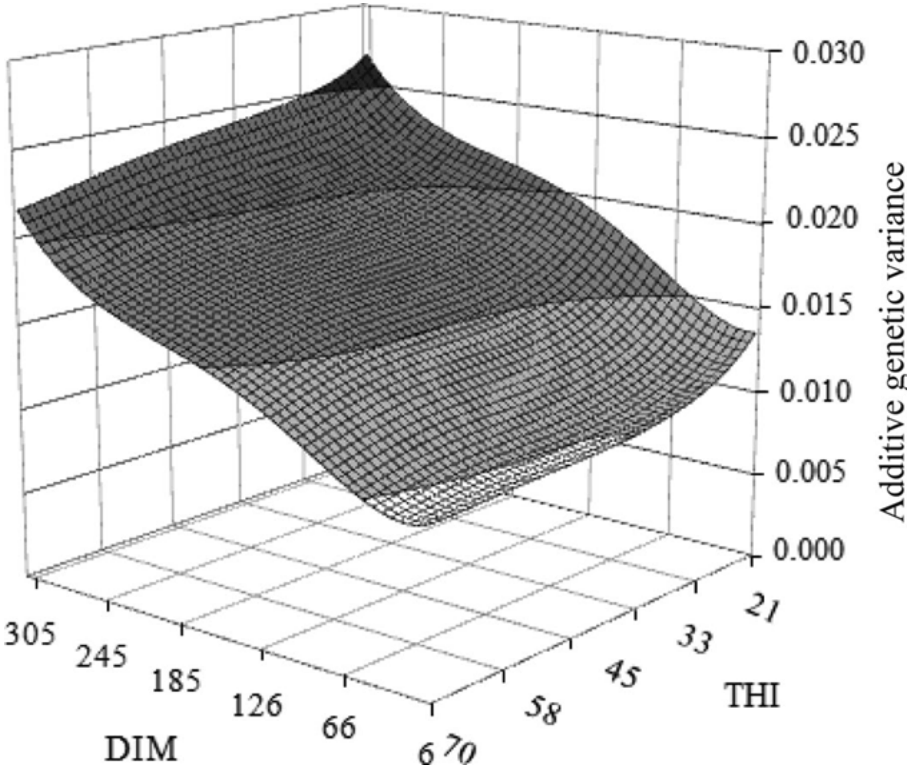
- Examine the relationship of THI and milk production- application of Random-Regression-Models (RRM); time dependent covariate **“days in milk” (DIM)** plus a continuous environmental gradient **“temperature-humidity index” (THI)**

- Allows the estimation of genetic parameters and breeding values for the whole grid of DIM x THI combinations (additional indications for cold stress effects)

- Allows to prove possible genotype x climate interactions

- Allows the identification of robust sires with stable and favorable breeding values across THI

Genetic Parameters for Clinical Mastitis by THI

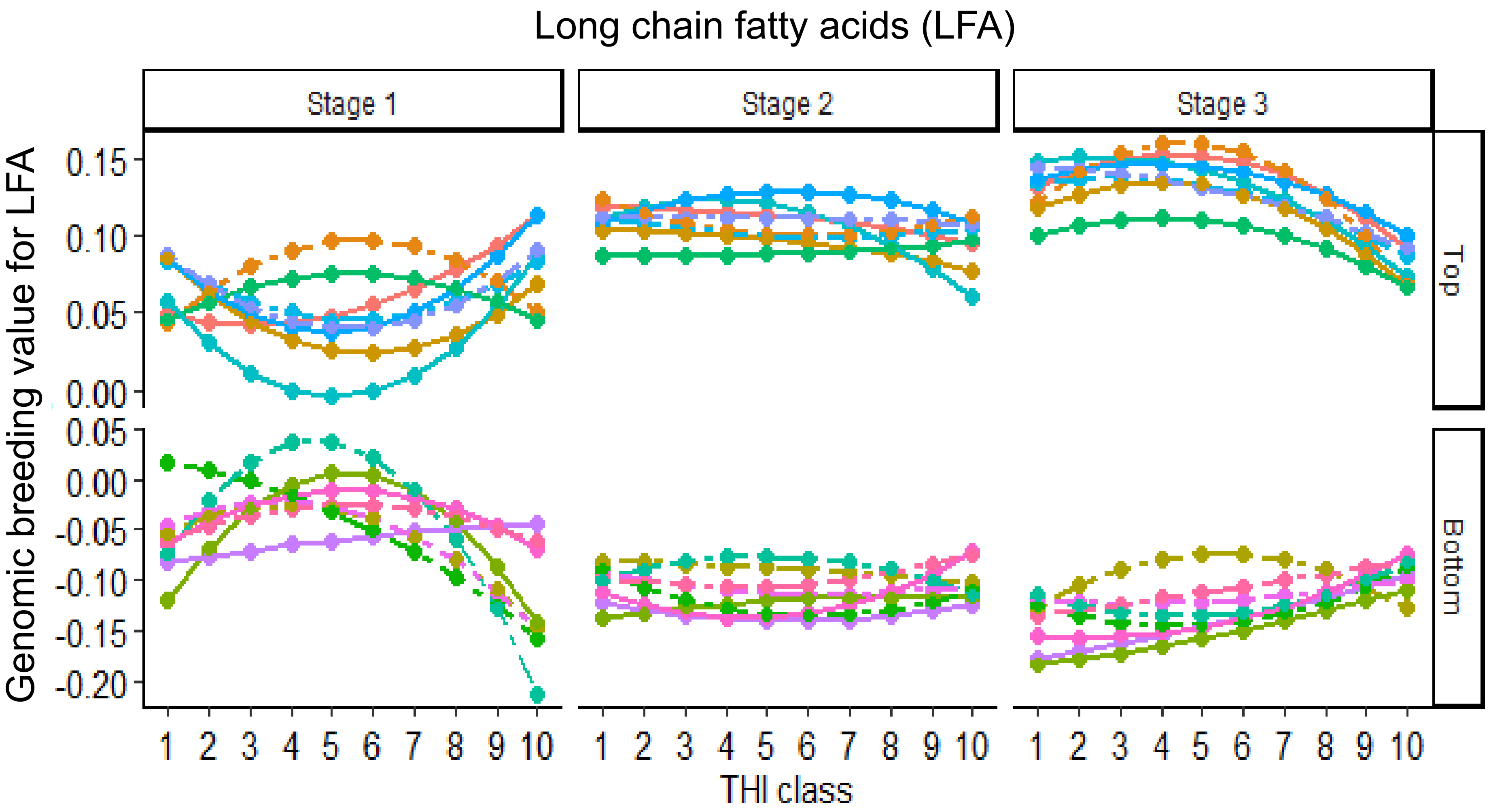


- Smaller genetic variances and genomic heritabilities for clinical mastitis with increasing THI (heat stress) and decreasing DIM (early lactation or metabolic stress)
- Clearer genetic differentiation under „comfort conditions”
- Background: Different genes are „switched on“ or „switched off“ with changing climate

Genomic Breeding Values for Clinical Mastitis by THI

„**Robust bulls**“:
Stable and high genomic breeding values for all THI
Minimize environmental effect

Most Sensitive Heat Stress Health Biomarker: Fatty Acids in Milk



Top-bulls
according to net merit

Bottom-bulls
according to net merit

New Phenotype- HSR

Definition of a new phenotype **H**eat**S**tress**R**esponse = **HSR**

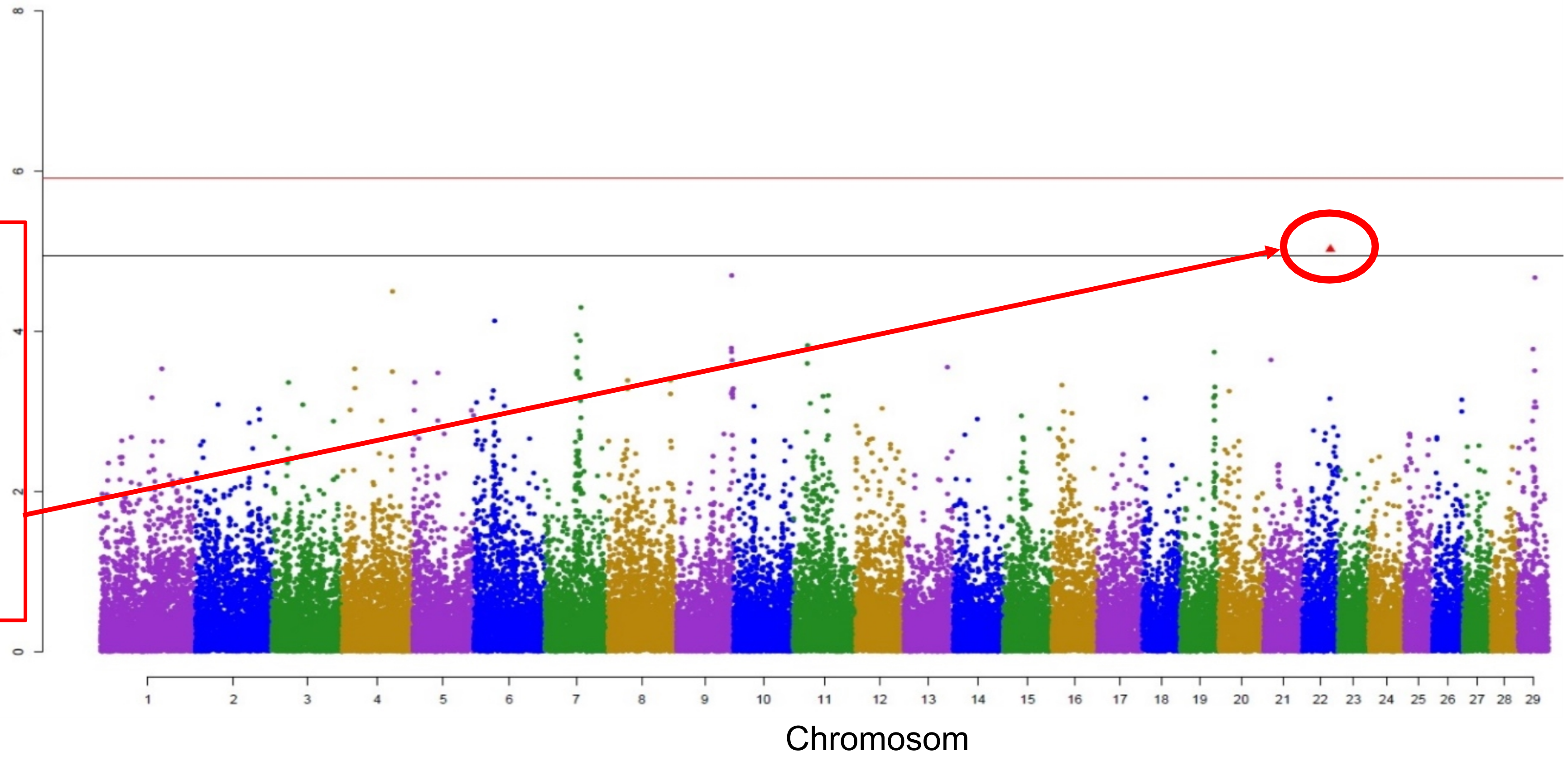
HSR = Genomic breeding value for **mastitis at THI 65** in relation to the breeding value for **mastitis at THI 35**

➔ Increasing values for HSR indicate animal robustness against heat stress

Genome-Wide Associations with Interaction Effects for HSR

➔ HSR was associated with dense genotype data and genome sequences

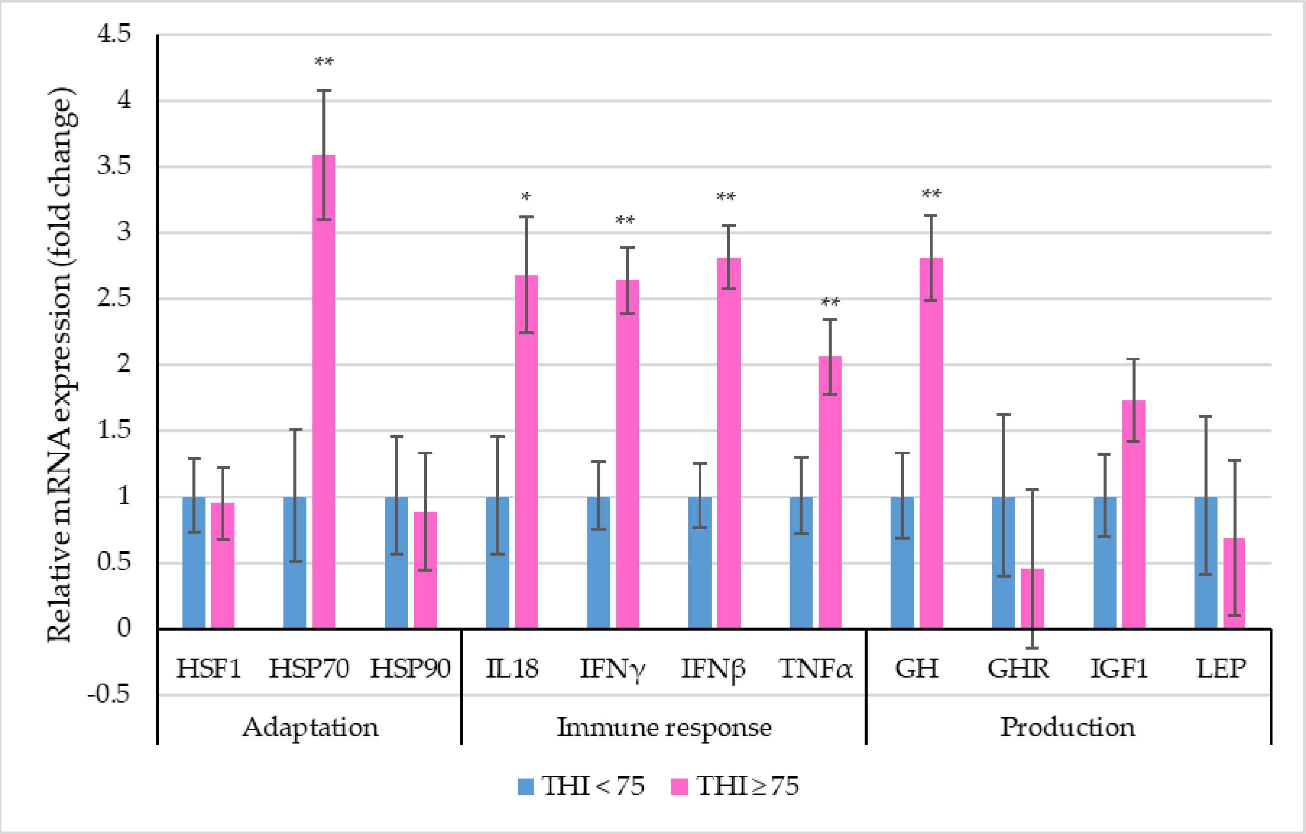
Software package *GWASInter.R* (Halli et al., (2022), PlosOne)



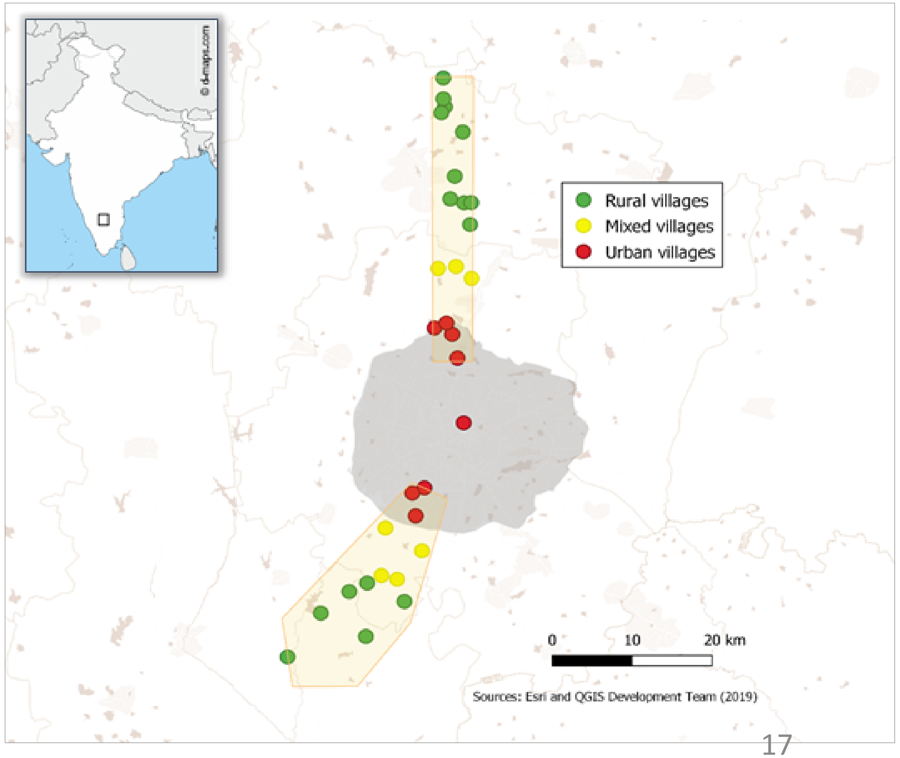
- SNP ARS-BFGL-NGS-16330 on **chromosome 22** located in the gene ***HEMK1***
- rats, mice, human: important role in immune functions, but depending on the **environmental conditions**

Studying the Way from Genotype to Phenotype: Gene Expressions

Focus on a really **heat stress challenging environment**:
The **megacity Bangalore** in Southern India
DFG-research unit FOR 2432/1/2/3: “Social-ecological systems”



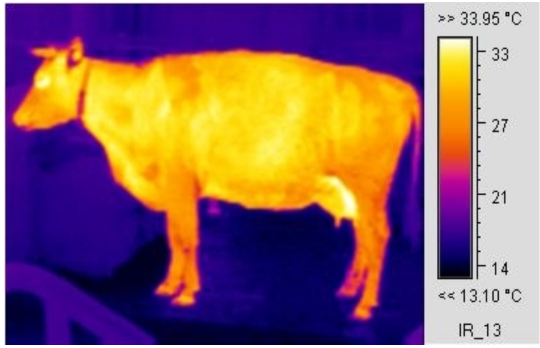
(Velayudhan et al., (2023), *Biology*)

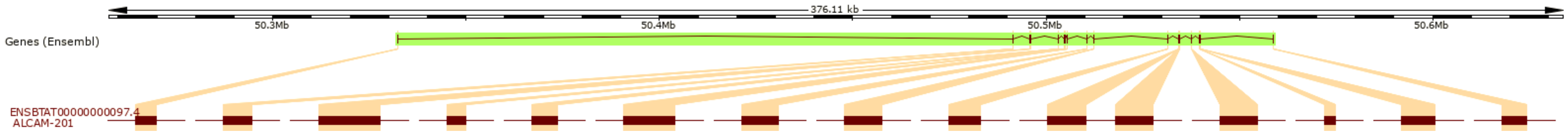


Sources: Esri and QGIS Development Team (2019)

Selective Sequencing of Holstein Cows in Bangalore for *HEMK1*

Selection of cows with extreme phenotypes for physiological traits for “**selective sequencing**”
(Heat images, respiration rate, body temperature, blood metabolites)



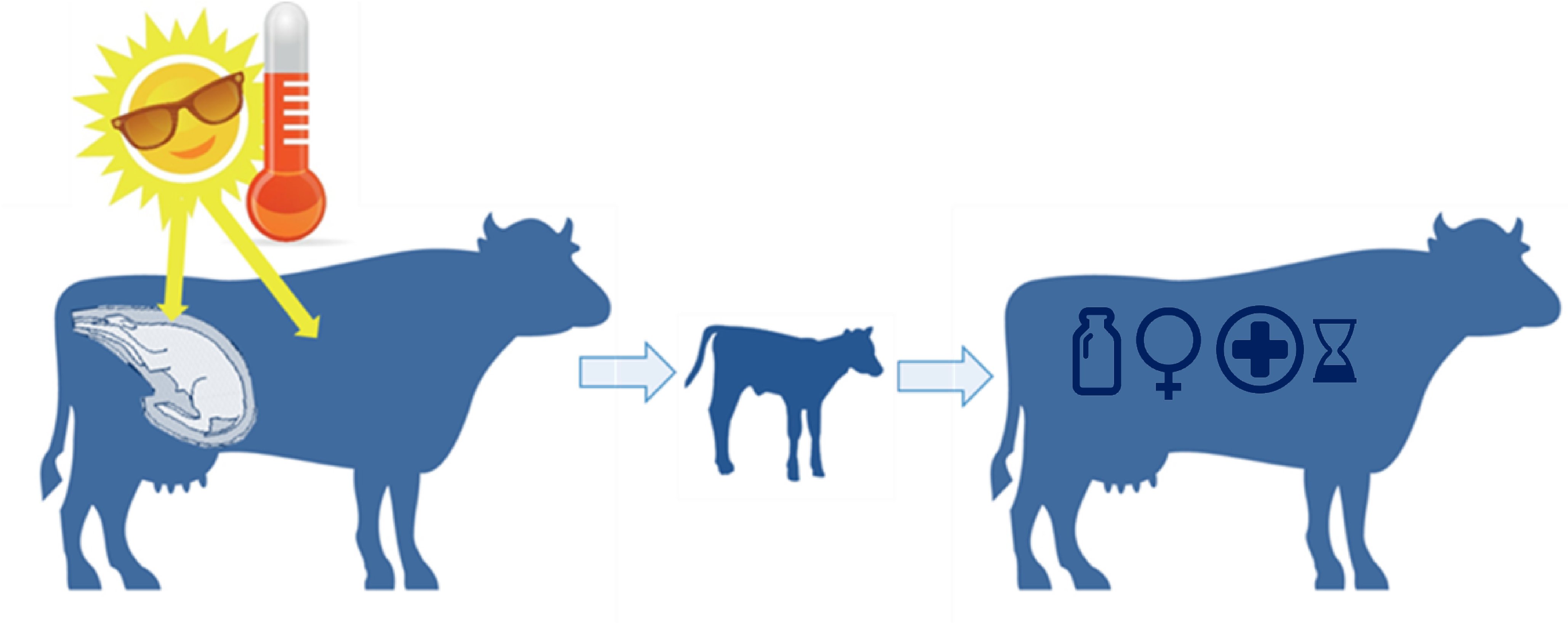


Sequencing of all 15 exons for a sample of 32 extreme heat resistant cows

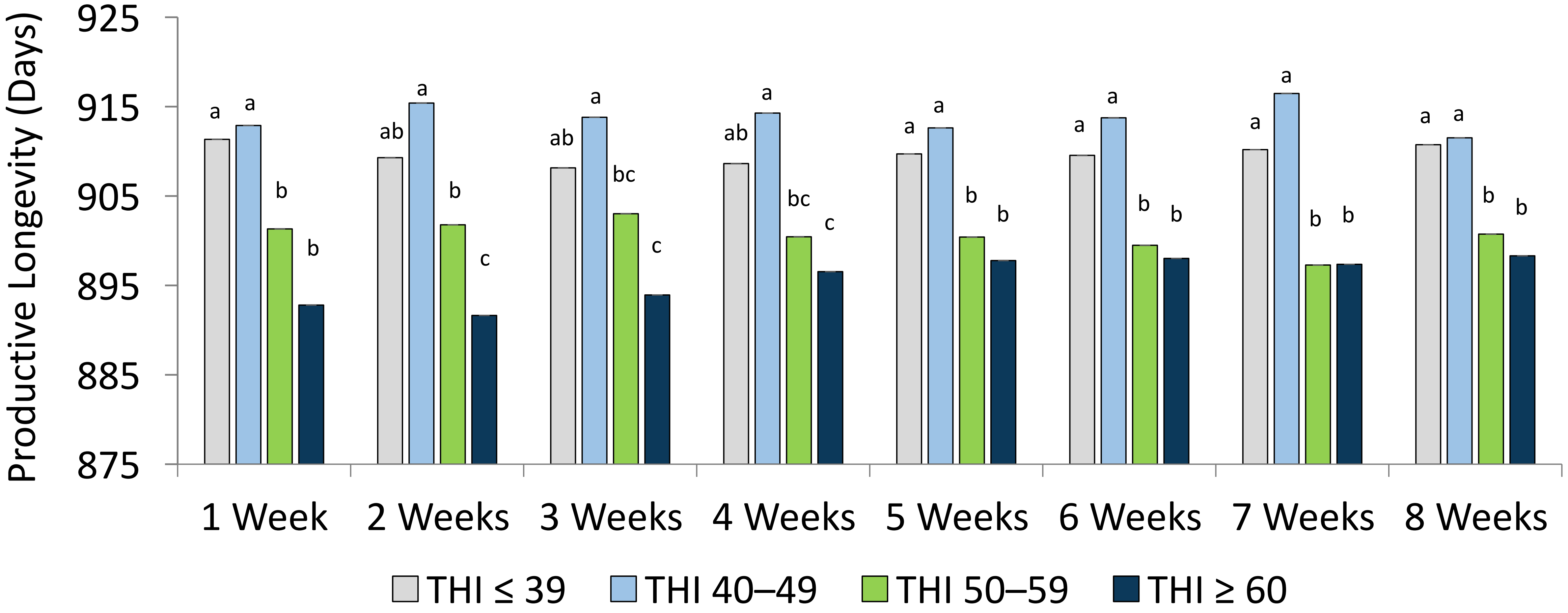
<u>SNP rs209844998 located in exon 9 displays an interesting genotype distribution:</u>

- 28 cows with **genotype AA**
- only **4 heterozygous** cows
- **no** genotype **TT**

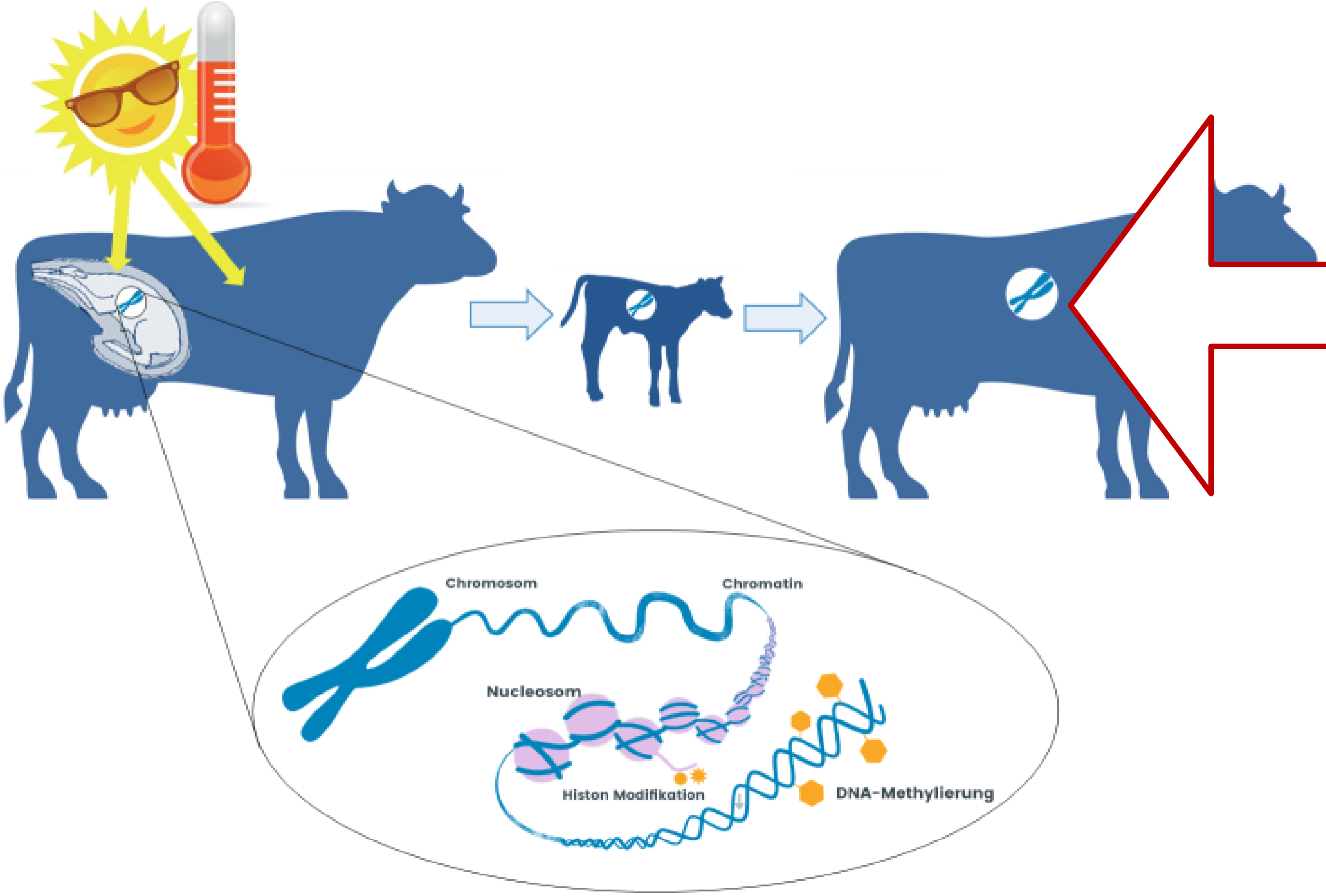
Heat Stress Effects Across Generations



Productive Longevity of Offspring in Relation to Gestational Heat Stress



Explanations for Genetic Effects Across Generations: Epigenomic Effects??



Can histone modifications and DNA methylations be transmitted from parents to offspring (F1 and F2-generations)?

Which chromosome segments are affected?

These are the research questions of the project “Multi-omics analyses of intra-uterine heat stress considering metabolomics and epigenetics”

Metabolome and Epigenetic Analyses: the Research Herds

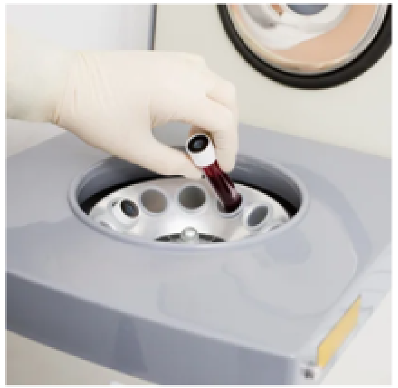
„Gladbacherhof“, research station of JLU Gießen
New cow barn including 2 sub-herds since April 2022;
Herd A: 60 HF-cows „high Input“
Herd B: 60 HF-cows „low input“
Modern trait recoding technique: (AMS, methane, dry matter intake, body weight, ketosis monitoring, **climate conditions, metabolome data, genome sequences**)

„Hofgut Neumühle“, research station for animal husbandry of Rhineland-Palatinate
Modern trait recoding technique: (methane, dry matter intake, body weight, ketosis monitoring, **climate regulations**, **metabolome data, genome sequences**)

- Blood sample collection of cows within 10 days before calving, and of calves within 7days after birth; the same design for the **F2-generation**
- 2 groups: HS in week 8 before calving / no HS
- Determination of 630 metabolites via MxP Quant 500 Kit (Biocrates Life Sciences AG) in cows and their female offspring

The “Pipeline” to Generate Metabolomics Data

Blood sampling



Centrifugation

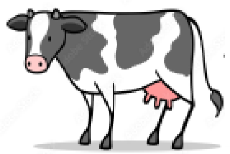
Plasma determination

Freezing at -80°C

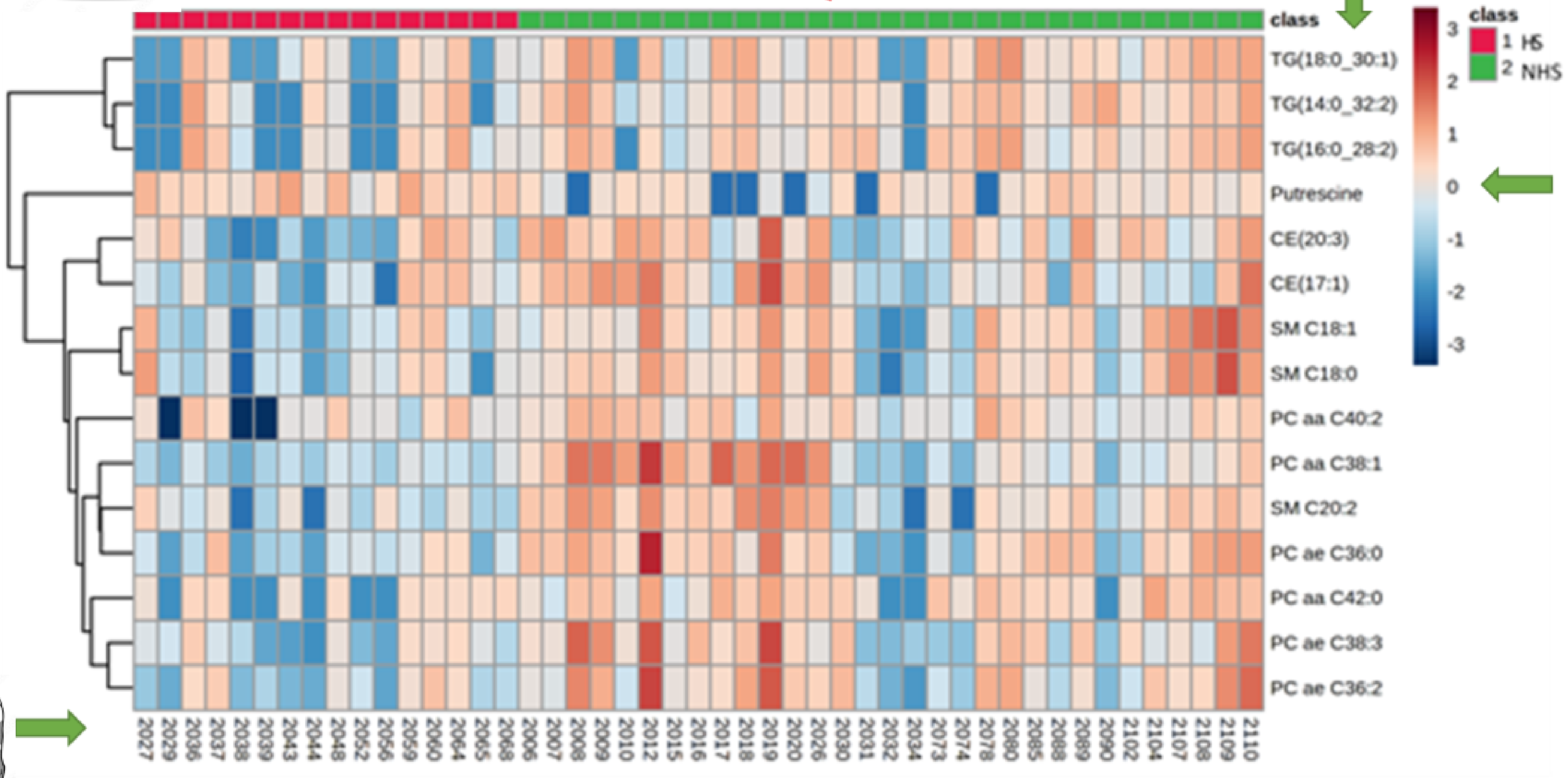
biocrates
The future of research and health

Determination of 630 different metabolites from 26 biochemical classes

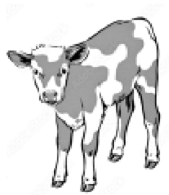
Heatmap: Maternal Heat Stress Significantly Effects Metabolites of Calves



Blood metabolites



Concentration (µM/L)



(Halli et al. (2023), *Metabolites*)

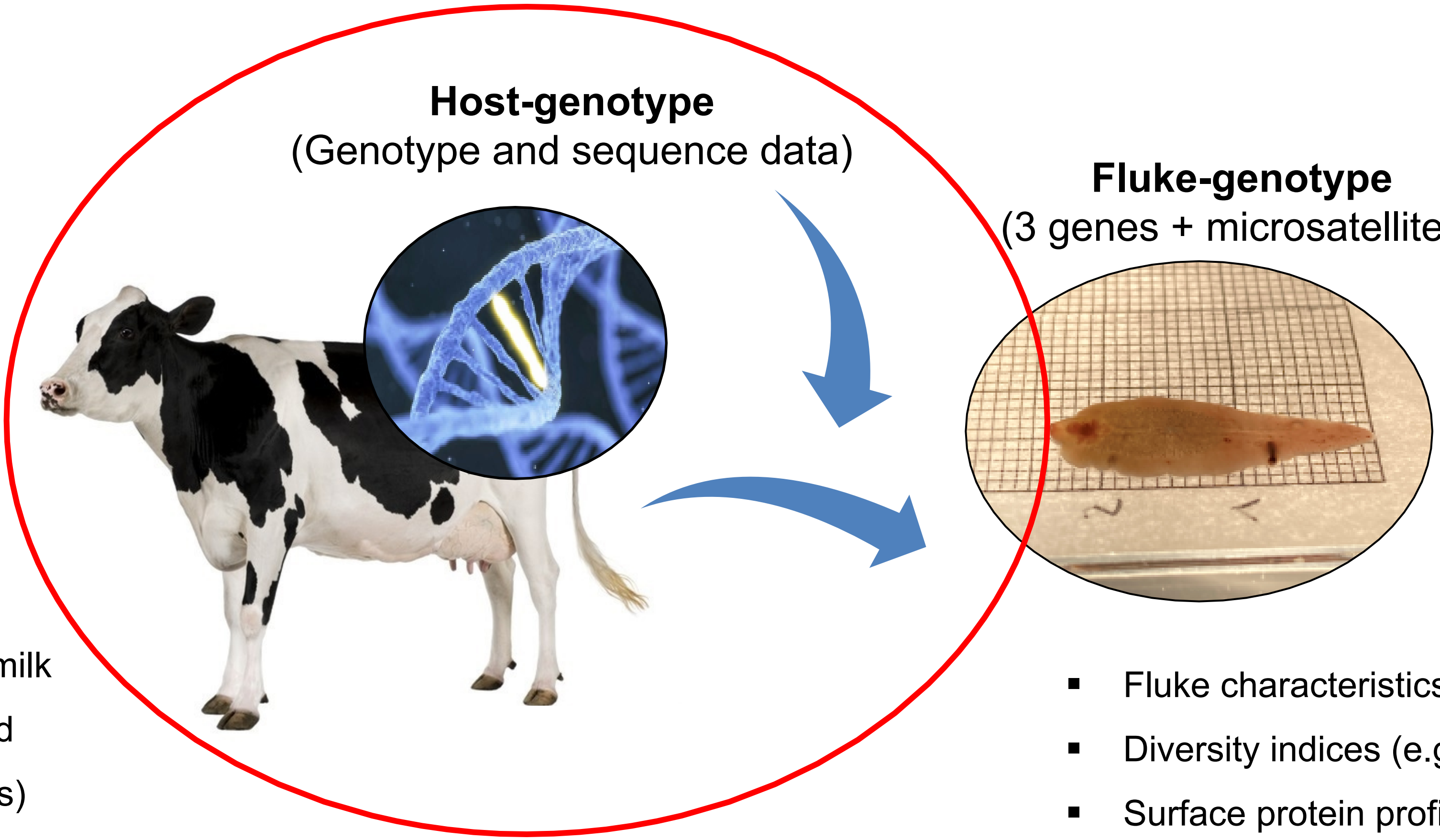
Cattle Health is Not Only the Genomics of the Host (Cow)……

- Genomics of the rumen microbiome (bacteria, protozoa, fungi, archaea)
- Genomics of gut microbiota
- Genomics of endoparasites (***Fasciola hepatica***, *Dictyocaulus viviparus (lungworms), rumen flukes*)

Research project in co-operation with veterinary science of TiHo Hannover:
“Host–parasite genotype (G_h x G_p) interactions and identification of genetic mechanisms underlying the host-parasite interface in **liver fluke (*Fasciola hepatica*)** infected dairy cows”

Central hypothesis: The interaction between host genotype and endoparasite genotype affects
a) the severity of the infection
b) the gene expression of the host
c) surface protein profiles of the pathogen

Phenotype and Genomics Data



Host-genotype
(Genotype and sequence data)

Fluke-genotype
(3 genes + microsatellites)

- Fecal egg count
- Antibody titre in blood and milk
- Liver enzyme levels in blood
- Liver damages (e.g., fibrosis)
- Fluke count in the liver
- Gene expression profiles in the liver

- Fluke characteristics (e.g., length)
- Diversity indices (e.g., Shannon-index)
- Surface protein profiles

The Current Challenge: Setting Up the G_h x G_p - Matrix

- To answer the question: which genotype combinations mostly affect gene expressions, egg counts, antibody titres and surface protein profiles?
- H - matrix: successfully developed ~10 yrs ago to combine host genotypes and host pedigree information

	Genotype of a specific host gene (e.g., *TLR5*)			Microsatellite genotype of the parasite		
	AA	**AB**	**BB**	**M1M1**	**M1M2**	**M2M2**
Cow A	1	0	0	0	0	1
Cow B	0	1	0	1	0	0
Cow C	0	1	0	0	1	0

- Ongoing process: construction of a symmetric matrix for host genotype x microsatellite genotype combinations with coefficients reflecting allele frequencies (“gene dosage”)

Conclusions: Strategies to Breed Robust Cows

1. Study breeding values of existing traits in relation to environmental alterations (e.g., climate, herd hygiene status, energy and protein in food,…..)
2. Improve health trait recording schemes ==> larger heritabilities ==> more accurate breeding values ==> increased response of selection
3. Create datasets simultaneously including novel traits, dense genotypes and environmental descriptors
4. Relate SNP significances and gene expressions in specific environments to physiological pathways and immunity measures
5. Consider the genotype and phenotype characteristics of pathogens in future research

Thank you for your attention!