vit IT-Solutions for Animal Production

65th EAAP Annual Meeting, 25-29 August 2014, Copenhagen / Denmark

EAAP Equine symposium:

Genomic research in horses in Europe

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EAAP - Horse Commission Symposium
Genomics and horse production: from science to practice

August 30th, 2012 Bratislava, Slovakia
8.30-18.00

Scope of this talk:

review & update
of the high-quality
scientific work
in the field of equine
genomics in Europe

Session 45. Genomics and horse production: from science to practice

Date: 30 August 2012; 08:30 - 12:30 hours
Chairperson: Mickelson and Von Velsen-Zerweck

Theatre	Session 45 no.
Genomic signatures of selection in the horse *Mickelson, J.R., Petersen, J.L., Mccue, M.E. and Valberg, S.J.*	1
A mutation in a novel transcription factor affects the pattern of locomotion in horses *Andersson, L.S., Schwochow, D., Rubin, C., Arnason, T., Petersen, J.L., Mccue, M.E., Mickelson, J.R., Cothran, G., Mikko, S., Lindgren, G. and Andersson, L.*	2
Genome-wide association mapping and genomic breeding values for warmblood horses *Distl, O., Metzger, J., Schrimpf, R., Philipp, U. and Hilla, D.*	3
First results on genomic selection in French show-jumping horses *Ricard, A., Danvy, S. and Legarra, A.*	4
The myostatin sequence variant g.66493737T>C detects evolution and domestication in horses *Dierks, C., Mömke, S., Philipp, U., Lopes, M.S. and Distl, O.*	5
Informative genomic regions for insect bite hypersensitivity in Shetland ponies in the Netherlands *Schurink, A., Ducro, B.J., Bastiaansen, J.W.M., Frankena, K. and Van Arendonk, J.A.M.*	6
Alternative splicing of the elastin gene in horses affected with chronic progressive lymphedema *De Keyser, K., Schroyen, M., Oosterlinck, M., Raes, E., Stinckens, A., Janssens, S. and Buys, N.*	7
Genetic variation in horse breeds derived from whole genome SNP data *Mickelson, J.R., Petersen, J.L. and Mccue, M.E.*	8
Genomic research in horses: the view of practitioners *Von Velsen-Zerweck, A. and Burger, D.*	9
Poster	**Session 45 no.**
Genomic selection in the Swiss Franches-Montagnes horse breed *Signer-Hasler, H., Flury, C., Haase, B., Burger, D., Stricker, C., Simianer, H., Leeb, T. and Rieder, S.*	10

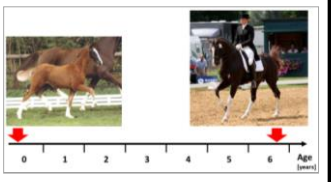
Background

- long tradition of horse breeding in Europe
- multiple breeds and studbooks
  - across and within countries
  - structural heterogeneity
  - intense exchange of genetic material (internationalization)
- breeding programs
  - routine assessments of breeding stock (foals, mares, stallions)
  - highly developed performance testing systems
  - genetic evaluations for conformation and performance traits

STRONG GENETIC RESEARCH IN HORSES IN EUROPE

Challenges

- spectrum of traits
  - late availability of phenotype information
    e.g. sport performance
  - limited access to reliable indicator traits
    e.g. stallion performance tests
  - lacking information on important phenotypes
    e.g. health



- approaches to speed up the genetic progress
  - shortening the long generation interval
    → earlier access to reliable predictions of genetic merit
  - increase of selection intensity for important traits
    → particularly for the challenging breeding goal traits
    (low h^2, difficult and limited access to phenotypes)

$$\Delta G = (i * r * \sigma_a) / L$$

with ΔG = genetic change, σ_a = genetic variability of the trait, i = selection intensity, r = accuracy of selection, L=generation interval (Falconer 1989)

high potential of using genomic tools in horses

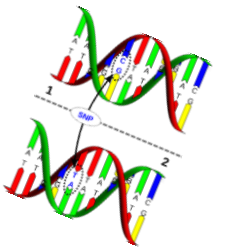
Research framework in Europe

- multi-center molecular genetic / genomic research in equids with relevant role in the worldwide genome research network
  - internationally recognized scientists and working groups
  - linking of expertise
  - efficient use of resources
- collaboration of science and industry as crucial factor
  - strong equine sector benefitting equine (genomic) research
  - optimum use of genomic tools
  - strategies for studying hereditary conditions

STRONG GENOMIC RESEARCH IN HORSES IN EUROPE

Areas of genomic research

- analyses of genome-wide genetic markers (BeadChip analyses)
- Sanger sequencing, Next Generation Sequencing (NGS)
- horse genome assembly (EquCab 3.0)
- population genomics
- genome wide association studies (GWAS)
- mutation analyses



Horse genome sequence

- **'The Horse Genome Project'**
  - international consortium:
    ≈100 researchers from > 20 countries
  - start in 1995, sequence publicly available in January 2007: **EquCab1.0**
- **EquCab2.0**
  - Whole Genome Shotgun (WGS) assembly at 6.8X
  - sequencing of individuals from multiple breeds
    → SNP library, 50k SNP chip
- **EquCab3.0**
  - high-throughput Illumina data (42X)
  - leverage of long and short read data (NGS) for new assembly

Basis of references genomes: 'Twilight' (top, photo: NHGRI), halfbrother 'Bravo' and 'Hrafnhetta'

SUCCESS OF COLLABORATIVE GENOMIC RESEARCH

Wade et al. (2009). Science 326, 865-867. Kalbfleisch et al. (2014). PAG XXII Conference, Jan 10-15, 2014, W279.
HGP website at http://www.broadinstitute.org/mammals/horse; UCSC Genome Bioinformatics at http://genome.ucsc.edu/

Genome sequence → genomic tools (I)

- key facts for further research
  - linkage disequilibrium (LD): moderate within breeds
    $LD_{human} < LD_{horse} < LD_{dog}$
  - impact of domestication
    differences between breeds / breed groups

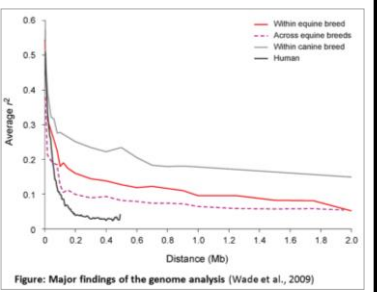


Figure: Major findings of the genome analysis (Wade et al., 2009)

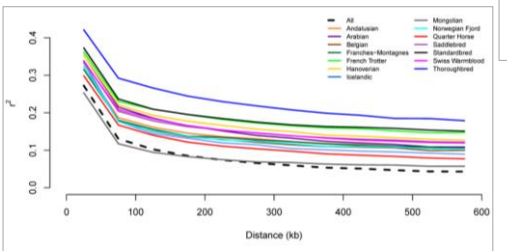


Figure: Decline in genome-wide linkage disequilibrium across and within breeds. (McCue et al., 2012)

Wade et al. (2009). Science 326, 865-867. Mikko et al. (2010). WCGALP, Aug 1-8, 2010, No.898. McCue et al. (2012). PLoS Genet 8, e1002451.

Genome sequence → genomic tools (II)

- key facts for further research
  - linkage disequilibrium (LD): moderate within breeds
    $LD_{human} < LD_{horse} < LD_{dog}$
  - impact of domestication
    differences between breeds / breed groups
- whole-genome SNP chips
  - Illumina 50k, 70k
  - Affimetrix HD (by the end of 2014)
- implications:
  - facilitated shift of research focus from monogenic to multi-/polygenic traits
  - perspective for routine applications in horse breeding

Power of gene mapping using the EqCab2 SNP chip
Wade et al., Science vol 326, 2009

Number of SNPs needed to differentiate horse haplotypes for within-breed gene mapping (by simulation)

Desired mean r^2max	0.7	0.8	0.9	1.0
High LD breed	30,000	100,000	175,000	245,000
Moderate LD breed	155,000	225,000	300,000	370,000
Low LD breed	250,000	320,000	390,000	460,000

Estimated from LD, number of haplotypes within haplotype blocks, and the polymorphism rate.
Source: Mikko et al. 2010

Wade et al. (2009). Science 326, 865-867. Mikko et al. (2010). WCGALP, Aug 1-8, 2010, No.898. McCue et al. (2012). PLoS Genet 8, e1002451.

Target traits

- conformation and functionality
  - body size and shape
  - conformational defects
- health and disease
  - skeleton and locomotor system
  - neurological function
  - immune system
  - metabolism
- performance
  - breeding
  - racing
  - riding

Photo: http://wwwwbfsh.org

BREEDING GOAL TRAITS IN THE FOCUS OF EQUINE GENOMIC RESEARCH

Conformation and functionality

Body size

- model trait for studying genetic influences on quantitative traits (within and across species)
- importance across breeds of horses
  - distinct types of equine skeletal morphology
  - specific body size and shape variation patterns within and across breeds
- recent increase of knowledge on genetic size regulation:

  LCORL (ligand-dependent nuclear receptor compressor-like protein)
  - consistent GWAS results in horses

    Franches-Montagne horses (Signer-Hasler et al. 2012)

    German warmblood horses (Kühn et al. 2012 / Tetens et al. 2013; Metzger et al. 2013a,b)

    Arabs (Ricard et al. 2013)

    multiple breeds and breed types (Metzger et al. 2013b)
  - relevant size regulator across species

    humans (Lango Allen et al. 2010), cattle (Pryce et al. 2011)

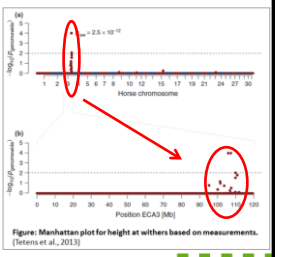


Figure: Manhattan plot for height at withers based on measurements. (Tetens et al., 2013)

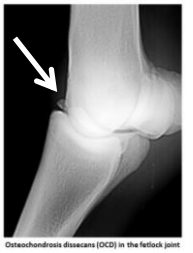
Health and disease

Skeleton and locomotor system

- major role of locomotor health for (long-term) usability of horses
- some conditions with significant prevalences across breeds, e.g. juvenile osteochondral conditions (JOCC) (Denoix et al. 2013)

osteochondrosis (OC)
- focal failure of enchondral ossification
- considerable impact on the market value of horses
- extensively studied worldwide for a long time (clinics, populations genetics, molecular genetics)
- multifactorial
- accumulating knowledge

  - faster now with the help of genomics


Osteochondrosis dissecans (OCD) in the fetlock joint

Health and disease / Skeleton and locomotor system

Osteochondrosis (I)

- importance of specific and reliable phenotype data!
- impact of trait definition

- 1,162 young **German WB** stallions
- radiographic protocols (binary OC/OCD)
- Illumina EquineSNP50® BeadChip (54,602 → 44,410 SNPs; 916 stallions)
- distinct results for hock and fetlock / toe, indications of major QTL on ECA20 (OCD fetlock / toe)

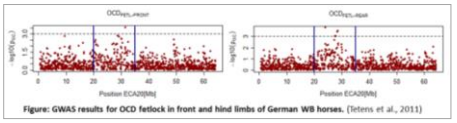


Figure: GWAS results for OCD fetlock in front and hind limbs of German WB horses. (Tetens et al., 2011)

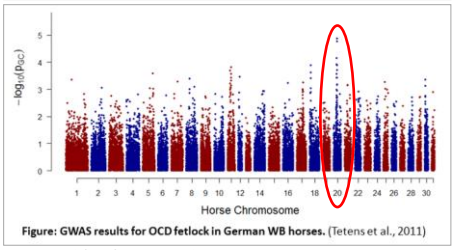


Figure: GWAS results for OCD fetlock in German WB horses. (Tetens et al., 2011)

Tetens et al. (2011). EAAP Annual Meeting, Starvanger, Norway.

- 583 **French trotters**
- radiographic protocols (scores, binary)
- Illumina EquineSNP50® BeadChip (54,602 → 41,249 SNPs)
- no evidence for major gene, no common SNP associations for hock OC (ECA3) and fetlock OC

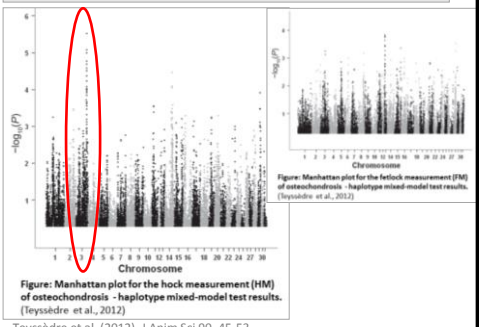


Figure: Manhattan plot for the hock measurement (HM) of osteochondrosis - haplotype mixed-model test results. (Teyssèdre et al., 2012)

Teyssèdre et al. (2012). J Anim Sci 90, 45-53.

Health and disease / Skeleton and locomotor system

Osteochondrosis (II)

- importance of specific and reliable phenotype data!
- impact of trait definition and study design

- QTL for major equine OC/OCD conditions reported on >20 chromosomes
- some chromosomes with >1 QTL
- confirmed and new QTL in recent GWAS (50k / 70k SNP chips)

Table: Genomic regions associated with osteochondrosis (OC) as shown by GWAS in Hanoverian Warmblood (HW), French Trotter (FT), Norwegian Trotter (NT) and English Thoroughbred (TB) horses. (Distl, 2013)

ECA	Breed	Trait	Position in Mb
1	HW	OC, OCD, OC-F, OCD-F, OC-H, OCD-H	43–45
2	HW	OC-F, OCD-F	104
3	HW	OC, OCD	64–65
3	TB	OCD	88
3	FT	OC-H	102–107
4	HW	OC-F, OCD-F, OC-H, OCD-H	41
5	NT	OCD-H	42, 77
10	NT	OCD-H	80
13	FT	OC-F	9–12
14	FT	OC-H	67–76
15	FT	OC-F	87–89
16	HW	OC-F, OCD-F	81
18	HW	OC, OCD	36
26	HW	OC-F, OCD-F	27
27	NT	OCD-H	38
28	NT	OCD-H	42
30	HW	OC-F, OCD-F	87

ECA = Equus caballus autosome; OC (OCD) = osteochondrosis (osteochondrosis dissecans) in fetlock and/or hock joints; OC-F (OCD-F) = osteochondrosis (osteochondrosis dissecans) in fetlock joints; OC-H (OCD-H) = osteochondrosis (osteochondrosis dissecans) in hock joints.
References: Corbin et al., 2012; Lampe et al., 2009; Lykkjen et al., 2010; Teyssèdre et al., 2012.

Distl, 2013. Vet J 197, 13-18.

Table: Quantitative trait loci (QTL) for osteochondrosis (OC) in Hanoverian Warmblood (HW) and South German Coldblood (SGC) horses. (Distl, 2013)

ECA	Breed	Phenotypic trait	Position in Mb
1	SGC	OC-F, OCD-F	161–182
2	HW	OC, OCD, OC-F, OCD-F, OC-H	19–37
3	HW	OCD, OCD-F	11–16
4	HW	OC, OC-F, OC-H	3–13
	HW	OC, OC-F	27–29
	HW	OC, OC-F	56–60
	SGC	OCD-F	50–66
5	HW	OCD-H	44–53
	HW	OC-F, OCD-F	76–93
	SGC	OC, OC-F	53–57
13	SGC	OCD-F	4–7
15	HW	OC-H	63–64
15	SGC	OC, OC-H	18–39
16	HW	OC, OCD, OC-F	6–24
	HW	OCD-H	33–45
	SGC	OC-F	23–28
17	SGC	OC, OC-F	41–42
18	HW	OC, OCD-H	74–82
18	SGC	OC-H	66–75
18	SGC	OC-F, OCD-F, OC-H	37–52
19	HW	OCD	0–1
21	HW	OC-H, OCD-H	5–17
22	SGC	OC, OCD-F	14–15, 31–48
23	SGC	OC, OC-F	37
25	SGC	OC, OC-F, OCD-F	0–1
27	SGC	OC-F	12–15
28	SGC	OC, OC-F	9–11
31	SGC	OC-H	21–22

ECA = Equus caballus autosome; OC (OCD) = osteochondrosis (osteochondrosis dissecans) in fetlock and/or hock joints; OC-F (OCD-F) = osteochondrosis (osteochondrosis dissecans) in fetlock joints; OC-H (OCD-H) = osteochondrosis (osteochondrosis dissecans) in hock joints.
References: Dierks et al., 2007; Dierks et al., 2010a,b; Felicetti et al., 2009; Lampe et al., 2009; Wittwer et al., 2007; Wittwer et al., 2008.

Health and disease

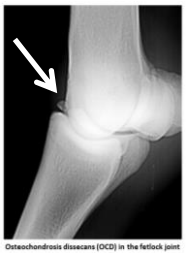
Skeleton and locomotor system (cont.)

- major role of locomotor health for (long-term) usability of horses
- some conditions with significant prevalences across breeds, e.g. juvenile osteochondral conditions (JOCC) (Denoix et al., 2013)

osteochondrosis (OC)

- focal failure of enchondral ossification
- considerable impact on the market value of horses
- extensively studied worldwide for a long time (clinics, populations genetics, molecular genetics)
- multifactorial
- accumulating knowledge:
  set of distinct conditions (several multigenic traits)

- OC and OCD in fetlock, hock and stifle joints
- fetlock: dorsodistal osteochondral fragments (DOFs)
- plantar osteochondral fragments (POFs) of hind limbs



Osteochondrosis dissecans (OCD) in the fetlock joint

- **importance of specific and reliable phenotype data!**
- **impact of trait definition**

Health and disease

Neurological function

- highly complex interplay of peripheral and central nervous function
  - some very specific functional defects
  - understanding of regular function (across species)
    often driven by studies of dysfunction (in target and model species)

Health and disease

Neurological function: Locomotion (I)

- highly complex interplay of peripheral and central nerval function
  - some very specific functional defects
  - **understanding of regular function** (across species)
    often driven by studies of **specific function** (in target and model species)

gaitedness and high-speed trotting

- breed-specific ability to perform alternate gaits,
  i.e. surplus to basic gaits (walk, trot, canter):
  pace, ambling gaits (regular rhythm, lateral, diagonal ambling)
- breed-specific ability to sustain trot at high speed,
  i.e. delay transition into canter
- related to general control / coordination
  of limb movement

Photo: Andersson, 2012

Health and disease

Neurological function: Locomotion (II)

- 70 **Icelandic horses**
  40 with pace (5-gaited), 30 without pace (4-gaited)
- 2 Icelandic horses
  1 with pace (5-gaited, AA), 1 without pace (4-gaited, CC)
- **capability of pace** (Y/N, competition records)
- Illumina EquineSNP50® BeadChip
  (54,602 SNPs), sequence data;
  mouse model (wild-type and Dmrt3-null mice)
- GWAS: strong association signal on ECA23
- mutation detection: nonsense mutation C/A
  (transcription factor DMRT3)
  → premature stop codon
  (truncated protein, 174 amino acids less)
- DMRT3 gene expression: subset of inhibitory
  neurons in spinal cord (crossing dorsal midline,
  connecting to motor neurons)

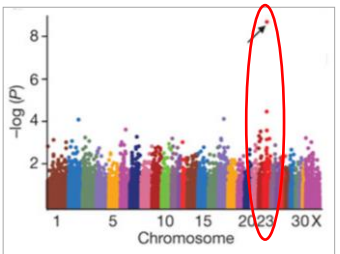


Figure: GWAS results for the ability to pace with genome-wide significant SNP on ECA23. (Andersson et al., 2012)

Table: Distribution of SNP genotypes. (Andersson et al., 2012)

	CC/CT	TT
pace	1	39
not pace	21	9

Andersson et al. (2012). Nature 488, 642-646. Andersson (2012). EAAP Annual Meeting, Bratislava, Slovakia.

Health and disease

Neurological function: Locomotion (III)

- ...
- 352 **Icelandic horses**,
  176 **gaited horses** (6 breeds),
  218 **non-gaited horses** (8 breeds),
  414 **horses bred for harness racing**
- high frequencies of DMRT3 mutation in gaited and harness racing horses
- DMRT3 mutation:
  permissive for performing alternate gaits, i.e. pace or four-beat ambling gaits, and high-speed trot ('gait keeper mutation')

Table: Distribution of the DMRT3 mutation in the Icelandic horse. (Andersson et al., 2012)

Phenotype	CC	CA	AA	Total
Five-gaited	0	1	65	66
Four-gaited	2	83	39	124
Total	3	105	149	257

$p = 2.4 \times 10^{-14}$

Table: Allele frequency of the DMRT3 nonsense mutation among horse populations. (Andersson et al., 2012)

Breed	*n*	*p*(A)
Icelandic horses*		
Four-gaited†	124	0.65
Five-gaited	66	0.99
Random sample	162	0.89
Other gaited horses		
Kentucky mountain saddle horse	22	0.95
Missouri fox trotter	40	1.00
Paso fino	45	1.00
Peruvian paso	19	1.00
Rocky mountain horse	17	1.00
Tennessee walking horse	33	0.98
Non-gaited horses		
Arabian horse	18	0.00
Gotland pony	28	0.00
North-Swedish draft horse	31	0.00
Przewalski's horse	6	0.00
Shetland pony	20	0.00
Swedish ardennes	22	0.00
Swedish warmblood	64	0.00
Thoroughbred	29	0.00
Horses bred for harness racing		
Standardbred, trotter (Sweden)	270	0.97
Standardbred, trotter (USA)	57	1.00
Standardbred, pacer (USA)	40	1.00
French trotter (France)	47	0.77

Andersson et al. (2012). Nature 488, 642-646. Andersson (2012). EAAP Annual Meeting, Bratislava, Slovakia.

Performance

Racing and riding


Photo: Ricard et al., 2012


Photo: http://wwwwbfsh.org

- performance criteria
  - mostly use-specific criteria in different horse breeds (breed formation, generations of human selection)
  - some characteristics of relevance in either discipline
- **'total picture':**
  locomotion pattern, physiological characteristics, behavior, ...

Performance

Racing and riding: racing

- performance criteria
  - mostly use-specific criteria in different horse breeds (breed formation, generations of human selection)
  - some characteristics of relevance in either discipline
- **'total picture':** locomotion pattern, physiological characteristics, behavior, ...

- 659 French **trotters**
- trotting race records (probability of qualification test, proportion of disqualified races, earnings)
- *DMRT3* genotyping; Illumina EquineSNP50® BeadChip
- *DMRT3*: no fixation of AA genotype, association of CC genotype with weaker performance (QT probability ↓, disqualification rate ↑, earnings ↓), intermediate results for CA genotype (QT probability ↓, earning of 5-years-olds ↑)
- GWAS: some stronger associated SNPs

Ricard et al. (2013). 10th Internat. Equine Gene Mapping WS, Azores, Portugal, July 10-13, 2013.

- 597 **Arabian endurance horses**
- endurance race records (speed, distance, rates of finished races)
- Illumina EquineSNP70® BeadChip (74k → 56,200 SNP)
- no significantly associated SNPs for speed, but 2 SNPs on ECA6 and 1 SNP on ECA7 for distance and finishing rates

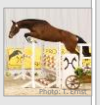
Ricard (submitted).

Performance

Racing and riding: riding

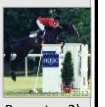
- performance criteria
  - mostly use-specific criteria in different horse breeds (breed formation, generations of human selection)
  - some characteristics of relevance in either discipline
- **'total picture':** locomotion pattern, physiological characteristics, behavior, ...

- 115 German WB stallions
- breeding values for jumping basis: scores for **free jumping**; BV range 56 - 171, reliability 0.87
- Illumina EquineSNP50® BeadChip (54,602 → 43,441 SNP)
- QTL on ECA1, 8, 9 and 26, further putative QTL on ECA 1, 3, 11, 17,217
- genotype-based breeding values: sum of additive and dominance effects of 6 SNPs explaining 54% of variance of conventional BV



Schröder et al. (2011). Anim Genet 43, 392-400.

- 908+102 **show-jumping** horses, 289 **3-day-event** horses (French sport horse, Anglo-Arabian, other European sport horses)
- deregressed breeding values for **competition** performance (basis: ranking and points, previously money earned)
- Illumina EquineSNP50® BeadChip (54,602 → 44,424 SNP for GWAS, 44,444 for GS)
- GWAS: no evidence of major gene, but for show-jumping suggestive QTL on ECA1 (Ryanodine Receptor 2)
- GS show-jumping (908 horses): only slight improvement of prediction accuracy (r^2_{convGE}=0.36, r^2_{GS}=0.39)



Ricard et al. (2012). EAAP Annual Meeting, Bratislava, Slovakia.
Ricard et al. (2013). J Anim Sci 91, 1076-1085. Brad & Ricard (subm.).

Target traits & genomic approaches

- increasing complexity of phenotypes
  implying increasing challenges (also) in genomic research
  - conformation and functionality
  - health and disease
  - performance
- <u>maximum use of the prospects of genomics</u>
  - **high standards for phenotyping**
  - efficient use of resources
    (samples, data, genomic tools, knowledge)

⇩

- recent development of genomic research in horses in Europe
  - several initiatives with joint engagement of science and industry
  - national and international consortium partners

Recent developments

Collaboration projects

Danish project on GS

- focus on **performance and health**
- national consortium
  (open for international collaboration)
- selection of ≈500 horses for genotyping
  - DNA availability, low average relationship
  - EBV of as high as possible accuracy
    competition, young horse performance, conformation (health)
- strategy: moderate + high density genotyping
  majority of horses 70k, few selected horses 670k;
  single-step procedure
- aim: inclusion of genomic information
  in regular breeding evaluations

Sponsors:
The Danish Advanced Technology Foundation
Asta og Jul. P. Justesens Fond
Dansk Varmblod
Dept. of Veterinary Clinical and Animal Sciences

Horsegene

- **heritable diseases** of horses with severe impact on the horse sector
- international consortium: BEL, SWE, UK, NL
- strategy: samples over several breeds, multiple genomic tools
  samples sizes: 900+900 for IBH, 300+200 for CPL, 600+600 for OC; high-density genotyping, pooled genome sequencing (cases/controls), sequencing of key ancestors and imputation
- aims: identification of genes and genetic markers, implementation of GS against disease susceptibility in breeding programs

SME partners
- British Equestrian Federation
- Koninklijk Fries Paarden Stamboek
- Svenska Islandshästförbundet
- Belgisch Warmbloed Paard
- Swedish Horse Board
- Koninklijke Maatschappij Belgisch Trekpaard
- Studfarm De Broomeursen
- Stal Groonoweg B.V.
- Ostra Greda Islandshästgard
- Van Haeringen Laboratories

Future of genomic research

- **expansion of collaboration in the equine sector in Europe**
  - strengthening of the whole sector
  - clearly less concerns of the horse industry
    to join efforts in the new field (genomics)
    than in the traditionally studbook-specific breeding issues

  ⇒ expected: joint European research activity in equine genomics ↑
- scenarios for international collaboration
  - exchange of genotypes
    genotyped sires used for breeding in several studbooks / countries
  - joint genomic evaluation
    exchange of genotypes, phenotypes and pedigree information

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Animal Production

Thank you!