Welfare from a breeding point of view: WFFS & other hereditary diseases in sport horses

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DNA-structure
- Chromosomes
- Double stranded DNA
- Genes
- Exons & introns

Traditional breeding goes genomic
- Visual inspection by breeder or judge
- Breeder experience
- Objective measures
- Multifactorial traits
- Monogenetic traits
- Mutations result in altered/nonfunctional protein
- Multigenic/multifactorial traits
- Find the biological function behind selected traits
- Marker assisted selection
- Genomic selection

Mutations
Random mistakes during DNA-duplication
Mutation = “Single Nucleotide Polymorphism” (SNP)
DMRT3 homozygous A/A standardbred

DMRT3 heterozygous C/A standardbred

Gait scores in AA or CA Standardbreds

Myostatin in Thoroughbreds

Cracking the code:
The Speed Gene Revealed

Jäderkvist et al 2015
Myostatin in thoroughbreds

"Loss-of-function" mutation

More and larger muscle fibres

Myostatin in other breeds

American Standardbreds are fixed for the wild type allele (T).
Allele freq of the "sprinter mutation" (C) in French Trotters is 1%, which means that 2% of them are carriers of the "sprinter mutation".

How common is a mutation?

Genotype frequency
At individual level in the population

Allele frequency (mutation freq)
At gene level in the population

Carrier frequencies

Calculate from a random sample of the population
When breeding and selection goes wrong

Random breeding
Strong selection

Inbreeding
Rapid breeding gain
Slow breeding progress

Polysaccharide Storage Myopathy – PSSM, Type 1 & Type 2

Type 1:
- Increased muscle glycogen conc and abnormal polysaccharide accumulation in skeletal muscle
- Glycogen syntase (GS) encoded by the gene GYS1, autosomal dominant or codominant
- Higher GS activity result in stiffness and limblness
- Present in more than 20 breeds
  - Most common in draft breeds of continental European breeds
  - Low allele frequencies in British drafts
  - 8% of warmbloods carry the mutation
  - Rare to none existing in light breeds as xx and ox

Type 2:
- Unknown gene
- 92% of warmbloods with PSSM
- Abnormal gaits and muscle pain
- Less likely to have tied-up
- Normal levels of muscle glycogen

Microphthalmia & anophthalmia

- One or both eyes missing or undeveloped
- Bi- or unilateral
- Probably autosomal recessive
- Many candidate genes
- Could be different mutations in different families
Speculations or facts

- Many breeders look at pedigrees and try to figure out who are carriers
- Carriers with one parent tested as non-carrier will turn the other parent into an obliged carrier

Warmblood Fragile Foal Syndrome (WFFS)

- Symptoms:
  - Skin lesions
  - Extremely fragile and thin skin
  - Friable and very loosely attached to the underlying subcutaneous tissue
  - Subcutaneous oedema
  - Hematomas and seromas particularly in fetlocks and tarsus regions
  - Hyperextension of limb articulations
- Similar to HERDA
- Similar to Ehler-Danlos syndrome

Hereditary Equine Regional Dermal Asthenia – HERDA

- First case reported in 1971
- 95% of the cases are traced back to the stallion Poco Bueno, the other 5% to his father and brothers
- 14% carriers among Quarter horses
- Autosomal recessive
- Mutation in the gene cyclophilin B (PPIB) => defect collagen α-molekyl
- Similar to Ehler-Danlos syndrome in humans
Case in Switzerland 2010

- Swiss Warmblod with HERDA-symptoms but no mutation in the HERDA gene (PPIB)
- Excluded Ehlers-Danlos syndrome type IV, VI, VIIA, VIIB och VIIC

"Cutaneous asthenia" in a Warmblod foal (2011)

Degenerative Suspensory Ligament Desmitis

- DSLD (2011)
- Peruvian Paso and PP-crosses
- Until recently DSLD was considered to be a collagen defect limited to suspensory ligaments
- Systemic disease in tissues with high collagen content.
- Abnormal accumulation of proteoglycans also in superficial and deep digital flexor tendons, patellar and nuchal ligaments, aorta, coronary arteries and sclera in DSLD-affected horses

Degenerative suspensory ligament desmitis (DSLD)

- Equine Systemic Proteoglycan Accumulation (ESPA)

Ehler-Danlos affecting a Brasilian horse

- Mangalarga-Campolino cross
- WFFS N/N
- HERDA N/N
- New mutation?
- One of the Ehler-Danlos subtypes?

Ehler-Danlos syndrome in humans

- Overly flexible joints
- Stretchy skin
- Fragile skin
- EDS fetuses of asymptomatic mothers affected by premature birth, still birth and abortion

Ehler-Danlos syndrome

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Type</th>
<th>Codons affected</th>
<th>Impaired gene</th>
<th>Allele</th>
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</thead>
<tbody>
<tr>
<td>Marfan</td>
<td>COL5A1</td>
<td>Exons 14, 15</td>
<td>TGF-β</td>
<td>AD</td>
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<tr>
<td>Beurling</td>
<td>COL5A2</td>
<td>Exons 14, 15</td>
<td>TGF-β</td>
<td>AD</td>
</tr>
<tr>
<td>Ehler-Danlos (EDS)</td>
<td>COL6A3</td>
<td>Exons 1, 2</td>
<td>Collagen</td>
<td>AD</td>
</tr>
<tr>
<td>Arthrochalasia</td>
<td>COL1A1</td>
<td>Exons 1</td>
<td>Collagen</td>
<td>AD</td>
</tr>
<tr>
<td>Marfan-like</td>
<td>COL3A1</td>
<td>Exons 2, 3</td>
<td>Collagen</td>
<td>AD</td>
</tr>
</tbody>
</table>

WFFS is similar to EDS type VI, kyphoscoliotic type (kEDS, Nemo syndrome)

The WFFS gene

- Autosomal recessive
- PLD1 (lysyl hydroxylase 1)
- Cannot produce collagen (connective tissue)
- Old mutation
EDS in other species

- Cutaneous asthenia
- Dermatosparaxie


Do we need to panic?

Mutations in populations

Past: Much of the original variation is lost by time

Present: New variation added by mutation and migration

Carrier x Carrier cross

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>WFFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>NN</td>
<td>N/WFFS</td>
</tr>
<tr>
<td>WFFS</td>
<td>N/WFFS</td>
<td>WFFS/WFFS</td>
</tr>
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**Carrier x Carrier cross**

<table>
<thead>
<tr>
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<tr>
<td>WFFS</td>
<td>N/WFFS</td>
<td>WFFS</td>
</tr>
</tbody>
</table>

Dead foal

**How large is the risk to have a WFFS foal?**

10% x 10% = 1% risk to cross two carriers

1% x 25% = 0.25% risk to have an affected foal

**How large is the risk to have a WFFS foal?**

20% x 20% = 4% risk to cross two carriers

4% x 25% = 1% risk to have an affected foal
Carrier frequencies in USA
Veterinary Genetics Laboratory, UC Davis

<table>
<thead>
<tr>
<th>Breed</th>
<th>Number of tested horses</th>
<th>WFFS carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>KWPN</td>
<td>104</td>
<td>7%</td>
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<tr>
<td>Hannoveraner</td>
<td>76</td>
<td>20%</td>
</tr>
<tr>
<td>Holsteiner</td>
<td>42</td>
<td>7%</td>
</tr>
<tr>
<td>Oldenburger</td>
<td>22</td>
<td>9%</td>
</tr>
<tr>
<td>Rheinland-Pfalz-Saar</td>
<td>9</td>
<td>14%</td>
</tr>
<tr>
<td>SWB</td>
<td>7</td>
<td>0%</td>
</tr>
<tr>
<td>Trakhener</td>
<td>64</td>
<td>2%</td>
</tr>
<tr>
<td>Westphaler</td>
<td>7</td>
<td>14%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>340</strong></td>
<td><strong>9%</strong></td>
</tr>
</tbody>
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Few tested horses give unreliable frequencies.
Risk of WFFS cases in a population

The risk of picking two carriers in a population

The number of affected foals potentially born in a population of 4000 coverings per year

Frequency of carriers determine the potential number of affected foals

The frequency of the mutant allele in the population is approximately 1/2 of the carrier frequency.

Number of generations needed to reduce the frequency of a lethal mutation

<table>
<thead>
<tr>
<th>Original allele frequency</th>
<th>New allele frequency</th>
<th>Number of generations</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.25</td>
<td>2</td>
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<tr>
<td></td>
<td>0.1</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>98</td>
</tr>
<tr>
<td>0.1</td>
<td>0.05</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>90</td>
</tr>
<tr>
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<td>0.001</td>
<td>990</td>
</tr>
<tr>
<td>0.01</td>
<td>0.005</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>900</td>
</tr>
<tr>
<td></td>
<td>0.0001</td>
<td>9900</td>
</tr>
</tbody>
</table>

An undesirable mutation can quickly spread in the population

- If a well-used stallion is carrier
- If a carrier has positive characteristics
- At a low frequency, the mutation is hidden in the population and is discovered when the frequency increases
- Unfavourable mutations are usually lost
**Strategies in breeding**

- To consider:
  - How large is the population?
  - How large is the effective population size?
  - How large is the genetic variation in the population?
  - Is the population inbred?
  - How strong is the selection, i.e. how large proportion of the population is used in breeding?
  - Is the studbook closed or open?

- There is a risk to decrease the genetic variation for other traits if all carriers are excluded from breeding

- WFFS-status is one of many characteristics to consider when selecting breeding individuals

- Never ever cross two carriers!

- Prioritize noncarriers in further breeding from a carrier parent

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**Test and inform!**