



THE PACE MAKER OR THE GAIT KEEPER THE GENETIC KEY TO FLYING PACE AND NATURAL TOELT



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ABOUT ME



- Agronomist, master in Animal Science
- PhD student
- Department of Animal Breeding and Genetics, SLU
- Worked with this project since May 2013
- Very interested in horses, especially trotting
- Speaker at Gävletravet



GAITS IN HORSES

Walk



Trot



Canter



Tölt

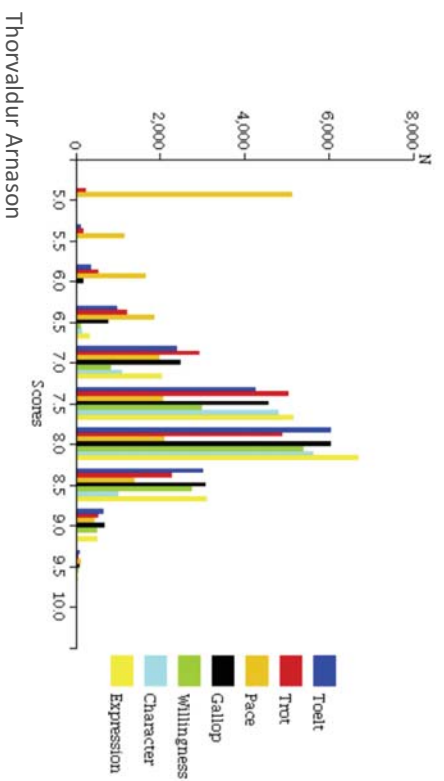


Pace



Photo: Freyja Innsland

Distribution of scores for riding ability IS 1990–2003



- 60 gaited breeds
- More in North- and South America
- Icelandic horse
- four- or five gaited
- A major discovery; DMRT3

MOLECULAR GENETICS – DIFFERENT TRAITS

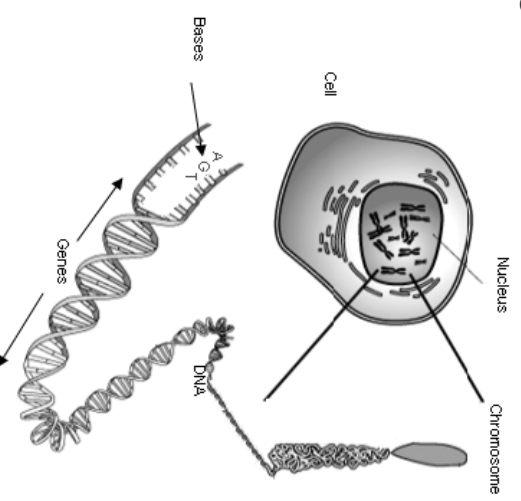
Monogenic traits: Determined by one gene and the environment has no or very small effect, e.g. coat color

Complex traits: Determined by a combination of many genes and the environment, e.g. temperament



MOLECULAR GENETICS - DNA

- DNA is a very long molecule that is shaped like a spiral rope ladder
- Made of four different kinds of smaller molecules (A,C,G,T)
- 32 pairs of chromosomes incl. sex chromosomes
- Half of the chromosomes from mare respectively stallion
- Horse genome sequenced 2007 (2.7×10^9 bp)



MOLECULAR GENETICS - GENES

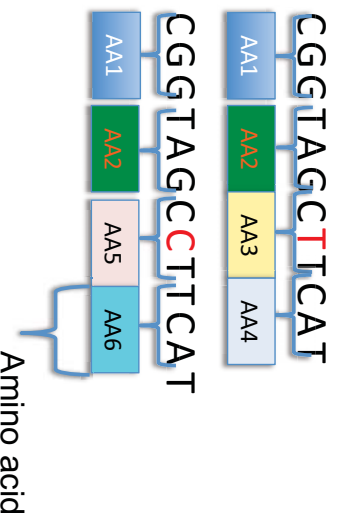
GENE: A fragment of DNA (ex. ATCCGCT) that code for proteins (~20 000 genes)

MUTATION: A change in DNA

PHENOTYPE: The traits of an individual

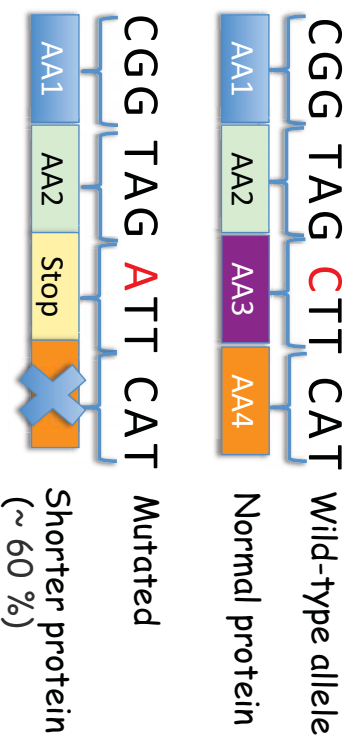
GENOTYPE: The genes of an individual

1 protein = > 21 amino acids



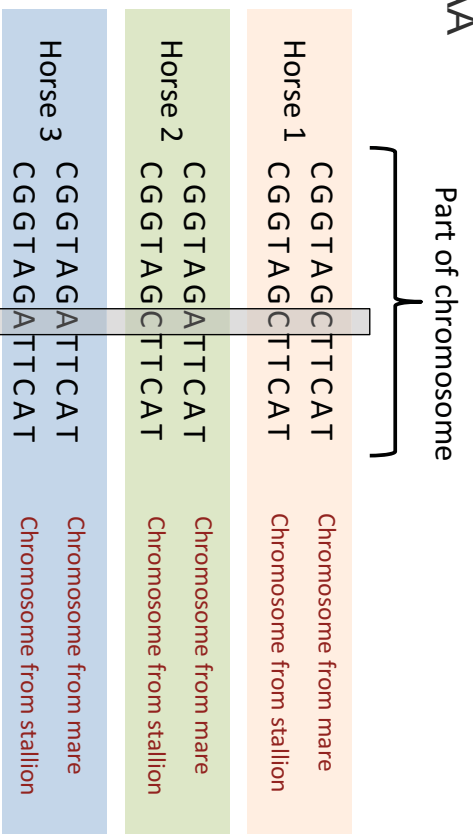
DMRT3 MUTATION

- C → A, Nonsense mutation
- Introduction of a stop codon
- Chromosome 23



METODOLOGY

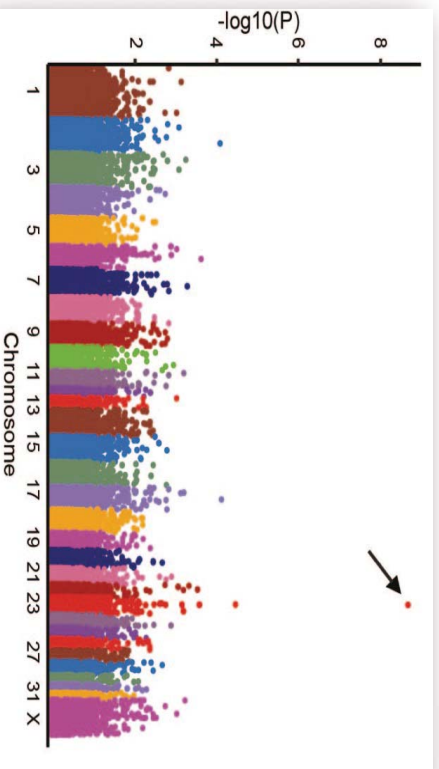
- It all started with a study about summer eczema
- “Scan” of the horses DNA, 50 000 SNPs
- SNP= “reading point”, where chromosomes differs
- CC, CA, AA



Horses can be homozygous or heterozygous for the SNP

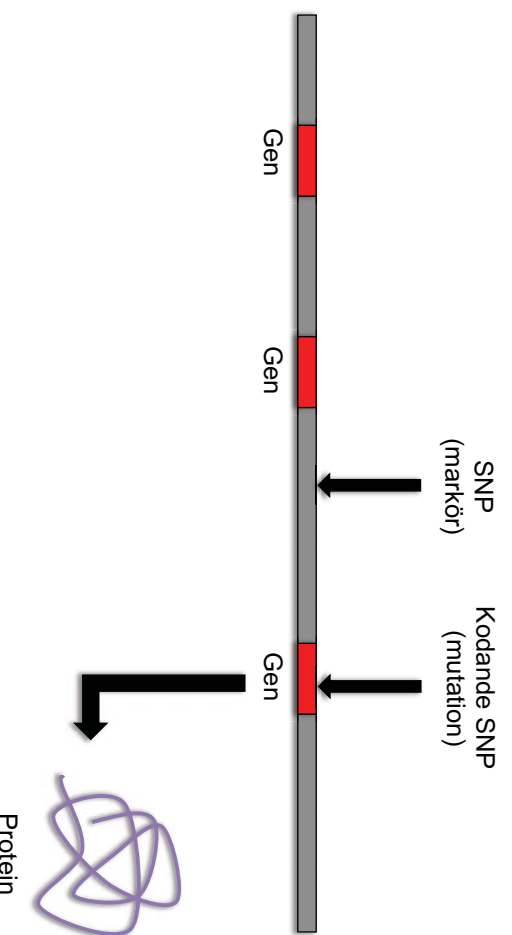
GENOME WIDE ASSOCIATION STUDY (GWAS)

- Tested 70 Icelandic horses
 - 30 four-gaited
 - 40 five-gaited (with pace)
- For each SNP we ask how well it fits that the four-gaited horses look genetically different from the five-gaited horses

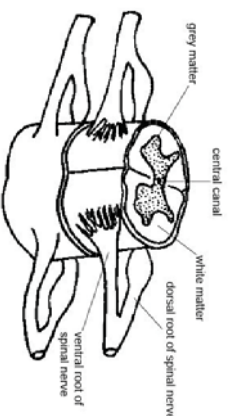


IDENTIFICATION OF THE CAUSING GENE

- The chromosome region was sequenced and one four-gaited horse was compared with one five-gaited
- Found the mutation that introduces the stop-codon
- $C \rightarrow A$
- CC, CA, AA



MUTATION IDENTIFICATION



- Gene with previously unknown function
- The gene is expressed in a subset of cells in the spinal cord
- These cells cross the dorsal midline of the spinal cord and connect the right and left hand sides of the horse
- Connect to motor neurons
- Influence the coordination of leg movement and coordination
- Mutation probably modifying the neuronal circuit in the spinal cord into a more flexible state

PACE

- Heritability for pace = 0.6 (+/- SE)
- Recessive trait, needs to be AA
- Almost all pacers are AA



DISTRIBUTION OF MUTATION IN ICELANDIC HORSES

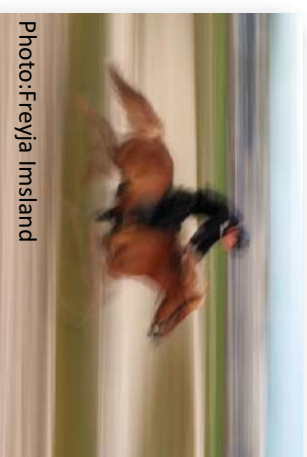


Evaluated horses genotyped for the mutation (replication)

PHENOTYPE	CC	CA	AA
Five-gaited	0	1	65
Four-gaited	2	83	39

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

- All but one five-gaited AA – maybe a miss-classification
- Most four-gaited horses CA
- 31% of four-gaited horses should be able to pace according to the genetic model (modifier loci or training)
- Low number of homozygous CC - consequence of breeding traditions?



EXAMINATION OF ICELANDIC HORSE PEDIGREES



MATING	NUMBER	% 4G EXPECTED	% 4G OBSERVED
none-pace x none-pace	336	75	75
none-pace x pace	1098	50	46
pace x pace	948	0	9

	Mare CA	
Stallion CA	CC 25%	CA 25%
	CA 25%	AA 25%

	Mare CA	
Stallion AA	AA 25%	CA 25%
	AA 25%	CA 25%

	Mare AA	
Stallion AA	AA 25%	AA 25%
	AA 25%	AA 25%

PACE HAS A RECESSIVE MODE OF INHERITANCE

CC = four-gaited
CA = four-gaited
AA = permissive for pace

- The quality of the pace is determined by a combination of multiple genes and environment



IS THE A-VARIANT PRESENT IN OTHER BREEDS?

- Gaited horses have more than the three basic gaits
- Different types of ambling gaits
- Also present in trotters



EXAMPLE OF GAITED BREEDS:

Icelandic Horse
American Saddlebred
Campolina
Mangalarga Marchador
Missouri Fox Trotter

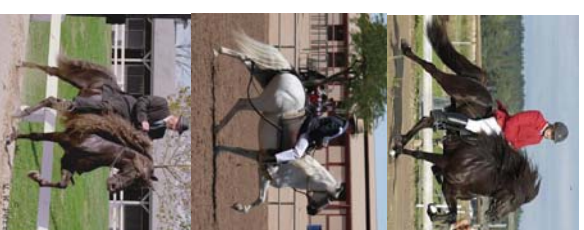
Peruvian Paso
Tennessee Walker
Racking Horse
Rocky Mountain Horse
Marwari Horse

Pacing Standardbred
Kentucky Mountain Saddle
Paso Fino
Virginia Highlander Horse
Albanian Horse

DISTRIBUTION OF MUTATION ACROSS BREEDS



BREED	CC	CA	AA
<u>Gaited</u>			
Icelandic Horse	3	105	149
Rocky Mountain Horse	0	0	17
Kentucky Mountain Saddle Horse	0	2	20
Missouri Fox Trotters	0	0	40
Peruvian Paso	0	0	19
Paso Fino	0	0	45
Standardbred Pacers	0	0	37
Tennessee Walkers	0	1	32
<u>Non-Gaited</u>			
Gotland Pony	28	0	0
Swedish Ardenne	22	0	0
Swedish Warmblood	35	0	0
Arabian	29	0	0
Thoroughbred	35	0	0
Shetland Pony	20	0	0
North Swedish Draft	31	0	0



HIGH FREQUENCY OF MUTATION IN "TROTTERS"



BREED	CC	CA	AA
Standardbred	3	31	304

- Blind study in a stable with 61 Standardbred horses
- Two horses were reported to have difficulties in sustaining trot
- Both were CA and the remaining 59 were AA



The mutation enables the horse to trot at fast speed without proceeding into gallop.

SELECTION AGAINST A IN SOME BREEDS?

- High frequency in gaited breeds and trotters but almost completely absent in most other breeds
- Seems to be unfavourable for horses used for show jumping, dressage, heavy drafting and gallop racing
- Four-gaited horses seems to be more adapted for dressage and more often win gallop races
- Five-gaited horses seems to be running very fast in trot
- The mutation has had a major impact on the diversification of the domestic horse



CONCLUSION



- Previously unknown molecule required for setting up the control centre coordinating limb movements in vertebrates revealed
- *DMRT3* nonsense mutation:
 - Causes the recessive trait pace in Icelandic Horses
 - Is permissive for a variety of alternate gaits across gaited breeds
 - Has a favorable effect on high speed trot in horses used for harness racing by delaying the transition to gallop



PRACTICAL IMPLICATIONS



AA horses

- Has the potential to perform flying pace*
- When mated to another five-gaited horse all offspring will be AA (inherits A to all its offspring).



CA horses

- Are very unlikely to perform flying pace
- Inherits A to 50% of its offspring, the rest inherits C



CC horses

- Are very unlikely to perform flying pace
- Can only produce four-gaited offspring (inherits C to all its offspring)

* Given other factors such as correct training, conformation and character.

PRACTICAL IMPLICATIONS

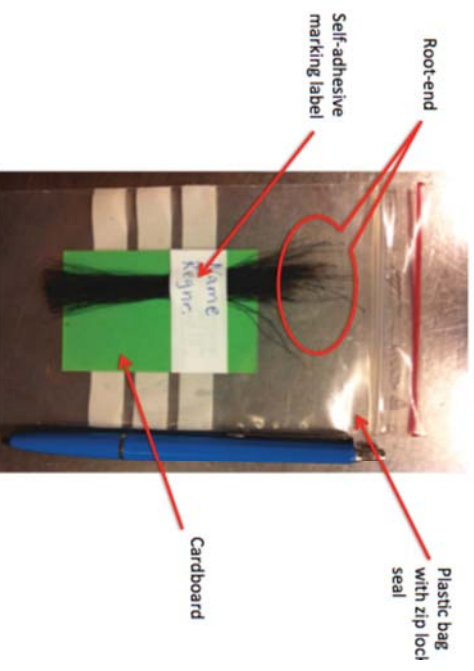


- DNA-test called *SynchroGait*® is available
- Genetic testing may be valuable for breeders and trainers of trotters and gaited horses world wide
- Aid in breeding schemes and training programs
- 10-15 working days, also a "quick-test"
- www.capiletgenetics.com



HOW DO YOU TEST YOUR HORSE??

- Similar procedure as the parentage control
- Simple hair sample
- The test is made at the laboratory at SLU
- With or without ID control
- More information can be found at the webpage
- All results are confidential



www.capiletgenetics.com

WHEN IS THE TEST INTERESTING

- Breeding
 - “Easier” to breed four-gaited horses
 - Does the four-gaited stallion that I want to use has the C-allele?
 - Did the foal inherit the C allele?
 - Preliminary data indicates that it might be wise to not breed CC horses
- Purchase
 - What type of horse do I want?
- Training
 - Guidance for how to train your horse the best, should you seek for pace?



Maybe this will change the breeding or the evaluation system of Icelandic horses?

MATINGS – A SIMPLE INHERITANCE PATTERN



stallion

	C:C	C:A	A:A
C:C	100% C:C	50% C:C 50% C:A	100% C:A
C:A	50% C:C 50% C:A	25% C:C 50% C:A 25% A:A	50% C:A 50% A:A
A:A	100% C:A	50% C:A 50% A:A	100% A:A



mare

IMPLICATIONS FOR WORLDFENGUR



- Work by Thorvaldur Árnason
- Genotype probabilities will be computed for all horses and this will be included in the “Mate selection program”
- Test development version on: www.ihbc.se or directly on www.ihbc.se/app/servlet/IsPedBLUPFItpacePB
- Horses with test for the DMRT3 genotype can be registered in WF

LOOKING FOR HORSES



- Icelandic horses for a study about tölt. Does *DMRT3* affect how difficult the horses are to put into tölt?
- Trotting horses used for riding



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THANK YOU FOR YOUR ATTENTION



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