Final Report Norwegian-Swedish Foundation for Equine Research, Stiftelsen Hästforskning

Swedish title: Kartläggning av prestationsegenskaper, genetisk variation och hälsa hos kallblodstravaren

English title: Mapping performance, genetic variation and health in the Coldblooded trotter

Project start: 2016-01-01
Project end: 2017-12-31

Major academic achievements based on this funding

Kim Fegraeus defended her PhD thesis “Exploring the horse genome to elucidate the genetics of gaits and athletic performance” on the 20th of October 2017. https://pub.epsilon.slu.se/14612/, Main supervisor: Gabriella Lindgren.

Brandon D Velie completed his post doctoral studies within this project from 2016-01-01 – 2018-08-31. Brandon now holds a permanent tenure-track position to become a professor in equine genetics and genomics at University of Sydney. Main mentor: Gabriella Lindgren. Co-mentor: Eric Strand.

Gabriella Lindgren is since February 2017 appointed as guest professor at KU Leuven, Livestock genomics group, Belgium.

Kim Fegraeus and Peter Kallings (SHF) at the promotion ceremony, SLU, fall 2018.

Project background

Historically the Nordic coldblooded horse (Lanthästen) has been the dominating horse breed in the Nordic countries for thousands of years. The modern Coldblooded trotter is derived from the Nordic coldblooded horse and has a wide range of high quality traits (bruksegenskaper) of value outside of the trotting arena. Only recently, since the 1950s, has it been more intensively bred for trotting performance even if the capacity for trotting has been present much longer. To investigate the impact of this push in selection we propose to study the genetic diversity of today's Coldblooded trotters. It will allow us to estimate the extent of inbreeding and to precisely monitor any change in genetic diversity in future horses. This is of particular importance as today's breeding mainly is dominated by the use of two stallion lines. As the Coldblooded trotter is intensively used in harness racing, another goal of this proposal is to investigate if there are particular genes linked to performance that have been selected for by breeders and therefore contributed to improved athletic performance and certain conformation traits in the breed. If so, it is of importance to monitor the future selection on these genes and any change in the overall genetic diversity. Strong artificial selection most likely not only resulted in an increased frequency of "good genes" in the breed, but also "less good" genes. For example, disease genes could have accumulated in situations where the breeding base is narrow. Unfortunately, this appears to be the case with certain airway disorders that have developed alongside the increased trotting capacity in the Coldblooded trotter. Upper respiratory tract (URT) obstructive disorders are a common problem among racehorses and are often associated with poor performance and respiratory noise during exercise. Because parts of the respiratory tract are formed as a tube, airflow resistance will be determined by its diameter. For example, a 20 % decrease in radius can lead to an approximate doubling of airway resistance. An obstructive disorder will therefore provide increased resistance to breathing and may cause dramatic clinical problems and hypoventilation. The disorders vary among individual horses within the same breed (Coldblooded trotters), but also among different horse breeds, hence genetic factors likely are involved in development of the disorders. A third goal of this study is therefore to perform a pilot study to pinpoint the genetic cause of dynamic laryngeal collapse, a very common upper respiratory tract obstructive disorder, in Coldblooded trotters.

List of objectives in research proposal

This SLU - NMBU collaborative study seeks to improve the performance, health and welfare of the Norwegian/Swedish Coldblooded trotter. Experts in Equine clinical veterinary medicine, performance evaluation and genetic testing will analyze the genome of this horse breed for: 1) race performance enhancing genes; 2) assess detrimental effects of inbreeding; and 3) genes that could predispose to dynamic laryngeal collapse (a very serious upper respiratory tract obstructive disorder which is very common in this breed of horse - relative to Standardbred trotters and Thoroughbred racehorses). Such genetic evaluation has the potential improve the health and sustainability of the Coldblooded racing trotter, and aids in setting objective goals regarding the breeding of these horses in Norway and Sweden. The project seeks to

also train a research associate for one year with the intension of this person becoming a graduate (PhD) student in the genetic assessment of Equine health & performance.

Specific aims in research proposal

1) To identify novel genes associated with athletic performance and conformation in Coldblooded trotters
2) To determine the genetic diversity and inbreeding in Coldblooded trotters
3) To perform a pilot study on mapping genes for the upper respiratory tract (URT) obstructive disorder “dynamic laryngeal collapse”. The results from this part of the proposal will serve as a foundation for a future larger genome scan for dynamic laryngeal collapse.

Status of project aims as of December 2018

Aim 1: To identify novel genes associated with athletic performance and conformation in Coldblooded trotters

Completed and published.

Velie BD, Fegraeus KJ, Solé M, Rosengren MK, Røed KH, Ihler CF, Strand E, Lindgren G. A genome-wide association study for harness racing success in the Norwegian-Swedish coldblooded trotter reveals genes for learning and energy metabolism. BMC Genet. 2018 Aug 29;19(1):80. doi: 10.1186/s12863-018-0670-3.

Scientific publication abstract:

Background. Although harness racing is of high economic importance to the global equine industry, significant genomic resources have yet to be applied to mapping harness racing success. To identify genomic regions associated with harness racing success, the current study performs genome-wide association analyses with three racing performance traits in the Norwegian-Swedish Coldblooded Trotter using the 670 K Axiom Equine Genotyping Array.

Results. Following quality control, 613 horses and 359,635 SNPs were retained for further analysis. After strict Bonferroni correction, nine genome-wide significant SNPs were identified for career earnings. No genome-wide significant SNPs were identified for number of gallops or best km time. However, four suggestive genome-wide significant SNPs were identified for number of gallops, while 19 were identified for best km time. Multiple genes related to intelligence, energy metabolism, and immune function were identified as potential candidate genes for harness racing success.

Conclusions. Apart from the physiological requirements needed for a harness racing horse to be successful, the results of the current study also advocate learning ability and memory as important elements for harness

racing success. Further exploration into the mental capacity required for a horse to achieve racing success is likely warranted.

Aim 2: To determine the genetic diversity and inbreeding in Coldblooded trotters

Completed and submitted for publication in Nov 2018.

Velie BD, Solé M, Jäderkvist Fegraeus K, Rosengren MK, Røed KH, Ihler CF, Strand E, Lindgren G (Under Review) Genomic measures of inbreeding in the Norwegian-Swedish Coldblooded Trotter. *Genet Sel Evol*

Scientific publication abstract:

Background: Since the 1950s, the Norwegian-Swedish Coldblooded trotter (NSCT) has been intensively selected for harness racing performance. As a result, a remarkable improvement in the racing performance of NSCTs has occurred; however, this improved racing performance has also been accompanied by a gradual increase in inbreeding levels. Inbreeding in NSCTs has historically been monitored using traditional methods based on pedigree analysis, but with recent advancements in genomics, the NSCT industry has indicated a desire to adopt more molecular approaches to the selection and maintenance of their breed. Consequently, the aims of the current study were to estimate genomic F coefficients for a sample of NSCT using a high density genotyping array and to compare these estimates to historical estimates of F within the breed.

Results: 566 raced NSCTs were available for analyses. Average genomic inbreeding coefficients ranged from 1.78% to 13.95%. Correlations between genomic inbreeding coefficients and pedigree inbreeding coefficients were significant ($P<0.001$) and ranged from 0.27 to 0.56. Comparisons of ROH between individuals yielded 1,403 regions that were present in at least 95% of the sampled horses. The average percentage of a single chromosome covered in ROH ranged from 9.84% to 18.82% with chromosome 31 showing the largest amount of homozygosity and chromosome 18 demonstrating the lowest amount of homozygosity.

Conclusions: Genomic inbreeding coefficients were higher than pedigree inbreeding coefficients with both methods showing a gradual increase in inbreeding levels in NSCTs between 2000 and 2009. Opportunities exists for the NSCT industry to develop programs that provide breeders with easily interpretable feedback on regions of the genome that are suboptimal from a genetic merit perspective or that are sensitive to inbreeding within the population. The use of molecular data to identify genomic regions that may contribute to inbreeding depression in the NSCT will likely prove to be a valuable tool for the preservation of genetic diversity in NSCTs in the long term.

Keywords (3-10): harness racing, homozygosity, horse, racehorse, runs of homozygosity

Aim 3. To perform a pilot study on mapping genes for the upper respiratory tract (URT) obstructive disorder "dynamic laryngeal collapse". The results from this part of the proposal will serve as a foundation for a future larger genome scan for dynamic laryngeal collapse.

Completed. Manuscript awaiting coauthors approval for submission in January 2019.

Velie BD, Smith PM, Solé M, Jäderkvist Fegraeus K, Rosengren MK, Røed KH, Fjordbakk CT, Ihler CF, Lindgren G, Strand E (Drafted manuscript: awaiting co-author final approval for submission) A pilot study exploring the genetics underpinning dynamic laryngeal collapse in Norwegian-Swedish Coldblooded Trotter racehorses. *Equine vet J*

Background: Dynamic laryngeal collapse associated with poll flexion is the most common upper respiratory disorder in the Coldblooded Trotter. Scientific studies performed at NMBU reveal this disorder to be among the most severe upper respiratory tract disorders in performance horses. The disorder is a great cause of wastage in Coldblooded trotters and has been the research topic of a previously NFR funded grant (202983). Dynamic laryngeal collapse seems to be caused by a combuination of certain phenotypes (anatomic characteristics) common in this breed of horse. The disorder is also commonly seen in other breeds of gaited horses. In this study a genome wide association analysis was performed in this breed using 33 affected horses and 26 unaffected airway controls.

Results: This study demonstrated that there is no correlation between "gaitedness" (DMRT3 gene) and Dynamic laryngeal collapse. Several genes were however identified which are candidates for further investigation which may be associated with development of this severe obstructive airway disorder.

Conclusions: We are getting closer to determining the genetic cause of this common disorder affecting the health and welfare of the Coldblooded Trotter.

Dissemination and publication list SHF

Oral presentations

- 2018. "Genetics and Genomics in Racing: A Champion Mindset". International Thoroughbred Breeders Federation (2018) Lexington, Kentucky, USA. (Brandon Velie)

- 181205. "Joint and tendon injuries – diagnosis, cause, treatment and prevention." To ca. 60 Equine enthusiasts/ riders/ trainers at Mjøsa Equine clinic, Stange, Norway. December 5, 2018. (Eric Strand)

- 181117. Upper respiratory tract obstructive disorders in horses: *from the perspective of a Specialist/ Researcher in Equine Surgery working in Northern Europe.* Annual Meeting of the Norwegian Equine Veterinary Association (130 veterinarians). Gardemoen November 17, 2018. (Eric Strand)

- 180918. Norwegian-Swedish breeding meeting. Results from the project were presented. (Christina Olsson)

- 181113. Coldblood meeing. Results from the project were presented. (Christina Olsson)

- 180921. ”Selektionsmönster i arvsmassan visar vägen till gener för travprestation”. Horse Research Day with Swedish Horse Industry, arranged by the Dept of Animal Breeding and Genetics. (Gabriella Lindgren)

- 181210. ”Genetics of gaits in horses”. Course Orthopedic Diagnostics, SLU. (Gabriella Lindgren)

- 181008. “Selektionsmönster i arvsmassan visar vägen till gener för travprestation”. Scientific mini conference, part of a course at SLU, Lecture room T, Ulls hus. (Gabriella Lindgren)

- 180129. “New tools for breeding equine athletes”. Resultat från kallblodstravarprojektet presenterades. A seminat in connection to Liesbeth Francois PhD defence on genetic diversity in horses. KU Leuven, Belgien. (Gabriella Lindgren)

- 180123. “Gångarter och Prestation hos Hästar – Vad styrs av generna?”. Two presentations at the vet school (NMBU) in Oslo (Kim Fegraeus).

- 180318. ”Aktuell genetikforskning på den Svensk-Norska Kallblodstravaren”. Presentation at Sleipners breeding seminar in Umeå. (Kim Fegraeus).

- 180407. ”Aktuell genetikforskning på den Svensk-Norska Kallblodstravaren”. Presentation at Sleipners breeding seminar in Sala. (Kim Fegraeus).

- 180914. “Exempel på molekylärgenetiska studier på häst”. Presentation at the course “The horse Biology” at SLU. (Kim Fegraeus).

- 180113. Using the unique breeding history of Coldblooded trotters to identify genes that influence athletic performance. SHFs coldblooded trotter project was also discussed. Plant and Animal Genome conference, San Diego, USA. (Gabriella Lindgren)

- March 2018. Presentation of the results from the inbreeding study to the breeding board for Swedish trotters. Christina Olsson (ST) arranged this meeting in Stockholm. (Brandon Velie)

- 2017. “Genetics and Genomics in Racing: Speed Isn’t Everything”. International Thoroughbred Breeders Federation (2017) Cape Town, South Africa. (Brandon Velie)

- 170113 – “Genetikens betydelse för hästars rörelsemönster - Att vara doktorand vid SLU”. Presentation for students at an Animal Breeding and Genetics course, SLU (Kim Fegraeus).

- 170330 – Presentation about the new *DMRT3* results, for the Swedish-Norwegian Coldblooded trotter committee, Solvalla, Stockholm (Kim Fegraeus & Brandon Velie)

- 170331 – “Genetiken bakom prestation och rörelsemönster hos travhästar”. Alumndag för hippologstudenter, SLU (Kim Fegraeus).

- 170906 – Seminar at the Swedish Trotting Association Breeding Conference. “Inbreeding in the Coldblooded trotter” and “En kartläggning av den svenska och norska kallblodspopulationen” (Kim Fegraeus & Brandon Velie)

- 170925 – Presentation for Swedish-Norwegian Coldblooded trotter committee. “Inbreeding in the Coldblooded trotter” and “En kartläggning av den svenska och norska kallblodspopulationen”. Arlanda, Stockholm (Kim Fegraeus & Brandon Velie)

- 170930 – Open house at SLU. “Gångarter och Prestation hos Hästar – Vad styrs av Generna?” (Kim Fegraeus)

- 171001 – Sluzewiec Racetrack, Warsaw, Poland. “Genetics and Genomics in Racing” (Brandon Velie)

- 171013 – Public presentation at Wångеn, Östersund during their Coldblooded trotter weekend. https://wangen.se/kallblodshelg/ (Kim Fegraeus)

- 171011. “New tools for breeding equine athletes”. Hästforskarträff, Equine Researchers meeting, SLU. Resultat från kallblodstravarprojektet presenterades. (Gabriella Lindgren)

- 170822. “New tools for breeding equine athletes”. From the horse´s perspective. Longines FEI European Championships, Gothenburg, Aug 22, 2017. (Gabriella Lindgren)

- 170713 – 170715. Dynamic Laryngeal Collapse associated with Poll Flexion: diagnosis, pathophysiology and treatment (Invited Speaker). *Proceedings: 5th World Equine Airways Symposium (WEAS)* p 65. Copenhagen, Denmark. July 13-15, 2017. Ca 200 Equine Vets/ researchers. (Eric Strand)

- 170922. “Understanding, diagnosing and preventing joint and skeletal problems in horses” 6 hours of lectures to Equine therapists at *Nordisk Heste- og Hundeterapiskole*. Brummendal, Norway. Sept. 22, 2017. (Eric Strand)

- 160702. Association of the mutation in the *DMRT3* gene with performance in Coldblooded trotters. Student VM i Ridsport, Flyinge. (Chameli Lawrence)

Media

Trav og Galoppnytt
http://www.slu.se/globalassets/ew/org/inst/hgen/diverse/trav-og-galopp-

nytt.pdf

Veterinärmagazinet
https://www.veterinarmagazinet.se/2017/06/effekten-av-travgenen-skiljer-sig-mellan-hastraser/

Hästsverige http://www.hastsverige.se/nyhetslista1.html?news=41641

Capilet Genetics, http://www.capiletgenetics.com/sv/nyheter

https://www.horsetalk.co.nz/2018/08/30/brains-brawn-success-harness-racing-horses/

Bra stam, bra träning - varför fungerar det inte? Inblick, Allt om Travsport.September 2018, sid 27-30.

https://www.horsetalk.co.nz/2017/05/11/gait-keeper-gene-cold-blooded-trotters-sweden-norway/

http://www.harnesslink.com/News/Scientists-Study-Coldblooded-Trotter-Career-Length

Scientists study coldblooded trotter career ::
Harnesslink
www.harnesslink.com

Fall 2018. Brandon Velie has made an interview with horse.com about the scientific article on “Racing longevity in the Swedish-Norwegian Coldblooded trotter” in Equine Vet Journal (2018). It will be published on horse.com during 2018.

Contributions to scientific conferences (talks, abstracts and posters)
Velie BD*, Bas Conn L, Petäjistö K, Røed KH, Ihler CF, Strand E, Fegraeus KJ, Lindgren G. The importance of MSTN for harness racing performance in the Norwegian-Swedish Coldblooded Trotter and the Finnhorse. Poster presentation delivered at the 11th World Congress on Genetics Applied to Livestock Production, Auckland, New Zealand: February 2018.

Jäderkvist Fegraeus K*, Velie BD, Olsson C, Lindgren G, Strand G, Ihler CF. Attrition rates and population parameters for the modern Coldblooded trotter. Poster presentation delivered at the Nordic Equine Veterinary Conference, Bergen, Norway: February 2018.

Jäderkvist Fegraeus, K, Olsson, C., Andersson, L., Ihler, C-F, Strand, E., Lindgren, G., Velie, B.D. Investigation of the association between the *DMRT3* "Gait keeper" mutation and early career performance in Coldblooded trotters. The 36th International Society for Animal Genetics Conference, Dublin, Ireland. 170716-170721

Jäderkvist Fegraeus, K., Velie, B.D., Olsson, C., Lindgren, G., Strand, E, Ihler, C-F. Racing longevity in the Swedish-Norwegian Coldblooded trotter. The Nordic Equine Veterinary Conference (NEVC), Bergen, Norway, 180208-180211 (Poster)

Popular science articles
Jäderkvist Fegraeus, K. Nyhetsbrev till "Framtidens djurhälsa och djurvälfärd-forskningsnyheter. 2017. Nr 6. Effekten av "travgenen" skiljer sig mellan hästraser

Jäderkvist Fegraeus, K., Strand E., Ihler, C.F., Lindgren, G. 2017. Nya forskningsresultat visar att effekten av "travargenen" *DMRT3* skiljer sig mellan kallblod och varmblod. Svensk Travsport, 2017-06-11. https://www.travsport.se/polopoly_fs/1.617882!/menu/standard/file/dmrt3%20hos%20kallblodstravare.pdf

Jäderkvist Fegraeus, K., Lindgren, G., Ihler, C.F. 2017. Travgenet påvirker ulikt hos kalblods- og varmblodstraver. Norsk-svensk hesteforskning, 2017-05-19. http://www.nhest.no/Nyheter/2017/Mai/Norsk-svensk-hesteforskning-Travgenet-pavirker-ulikt-hos-kaldblods-og-varmblodstraver/

Gabriella Lindgren. Gener för mental förmåga påverkar travframgångar. Framtidens djurhälsa och djurvälfärds nyhetsbrev (oktober) 2018. https://www.slu.se/forskning/kunskapsbank/publicerat/hastnotiser/gener-for-mental-formaga-paverkar-travframgangar/

Conference Manuscripts
Velie BD, Bas Conn L, Petäajistö K, Røed KH, Ilher CF, Strand E, Fegraeus KJ, Lindgren G. The importance of MSTN for harness racing performance in the Norwegian-Swedish Coldblooded Trotter and the Finnhorse. *Proceedings of the 4th World Congress on Genetics Applied to Livestock* (2018)

Peer-reviewed articles in international scientific journals

Velie BD, Smith PM, Solé M, Jäderkvist Fegraeus K, Rosengren MK, Røed KH, Fjordbakk CT, Ihler CF, Lindgren G, Strand E (Drafted manuscript: awaiting co-author final approval for submission) A pilot study exploring the genetics underpinning dynamic laryngeal collapse in Norwegian-Swedish Coldblooded Trotter racehorses. *Equine vet J*

Velie BD, Solé M, Jäderkvist Fegraeus K, Rosengren MK, Røed KH, Ihler CF, Strand E, Lindgren G (Under Review) Genomic measures of inbreeding in the Norwegian-Swedish Coldblooded Trotter. *Genet Sel Evol*

Todd ET, Jäderkvist Fegraeus K, Thomson PC, Ihler CF, Strand E, Lindgren G, Velie BD. Premie race participation is associated with increased career longevity and prize money earnings in Norwegian-Swedish Coldblooded Trotters. *Acta Agri Scand A Animal Sci, accepted for publication.* The DOI of the paper is: 10.1080/09064702.2018.1563211. https://doi.org/10.1080/09064702.2018.1563211 .

Velie BD, Fegraeus KJ, Solé M, Rosengren MK, Røed KH, Ihler CF, Strand E, Lindgren G. A genome-wide association study for harness racing success in the Norwegian-Swedish coldblooded trotter reveals genes for learning and energy metabolism. BMC Genet. 2018 Aug 29;19(1):80. doi: 10.1186/s12863-018-0670-3.

Velie BD, Jäderkvist Fegraeus K, Ihler CF, Lindgren G, Strand E. Competition lifespan survival analysis in the Norwegian-Swedish Coldblooded Trotter racehorse. Equine Vet J. 2018 Jul 3. doi: 10.1111/evj.12989.

Jäderkvist Fegraeus K, Lawrence C, Petäjistö K, Johansson MK, Wiklund M, Olsson C, Andersson L, Andersson LS, Røed KH, Ihler CF, Strand E, Lindgren G*, Velie BD*. Lack of significant associations with early career performance suggest no link between the DMRT3 "Gait Keeper" mutation and precocity in Coldblooded trotters. PLoS One. 2017 May 10;12(5):e0177351. doi: 10.1371/journal.pone.0177351. eCollection 2017. *Equal contribution.