Swedish University of Agricultural Sciences
Faculty of Veterinary Medicine and Animal Science

Association of the *DMRT3* nonsense mutation with pattern of locomotion in five different horse breeds

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Association of the *DMRT3* nonsense mutation with pattern of locomotion in five different horse breeds

Association av stoppmutationen i *DMRT3* med rörelsemönster hos fem olika hästraser

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SAMMANFATTNING

En stoppmutation i *DMRT3* genen har visats ha stor påverkan på hästars rörelsemönster. Hästar med gångarter utöver de vanliga; skritt, trav och galopp visar alternativa gångarter som tölt och/eller pass och är ofta hetero (CA)- eller homozygota (AA) för den här stoppmutationen medan hästar som inte har alternativa gångarter ofta är homozygota för vild-typ genen (CC). Till exempel kan Islandshästar utföra alternativa gångarter. Islandshästar som är femgångare kan gå i flygande pass och tölt, utöver de vanliga gångarterna skritt, trav och galopp, och är ofta homozygota för stoppmutationen (AA). Medan fyrgångshästarna som endast kan gå i tölt, utöver de vanliga gångarterna skritt, trav och galopp, ofta är heterozygota (CA) för stoppmutationen eller homozygota för vild-typ genen (CC). I denna studie har vi undersökt om stoppmutationen i *DMRT3* har signifikant betydelse för hur lätt Islandshästen är att töltsätta. Vi har även testat om varmblods- och kallblodstravare som inte har rätt genetiska förutsättningar till att prestera bra på travbanan lättare kan skolas om till ridhästar genom att de har en bättre galopp och om det beror på genotyp i *DMRT3*. Vi har även testat om stoppmutationen i *DMRT3* har betydelse för hur många gångarter Morgan och American Curly kan prestera. Studierna har inkluderat insamling av hårprover och fenotypiska beskrivningar genom frågeformulär. I denna studie har 263 hårprover analyserats. Det har visats att Islandshästar som är homozygota för vild-typ genen har svårare för att töltsättas än hästar som är hetero- eller homozygota för stoppmutionen i *DMRT3*. Varmblodiga travhästar som är heterozygota för stoppmutationen har visats ha lättare för att bli ridna i galopp och därigenom lättare bli omskolade till ridhästar än hästar som är homozygota för stoppmutationen. Stoppmutationen i *DMRT3* har visats ha stor betydelse för hur många gångarter Morgan och American Curly kan komma att prestera.

ABSTRACT

A nonsense mutation in the *DMRT3* gene has been shown to have a large impact on pattern of locomotion in horses. Horses that can perform several other gaits in addition to the normally occurring gaits, walk, trot and canter, are often hetero (CA)- or homozygous (AA) for this nonsense mutation. Horses that only can perform walk, trot and canter are often homozygous for the wild-type gene (CC). For example the Icelandic Horse is a gaited breed. Five-gaited Icelandic horses can perform both flying pace and tölt, except for the normally occurring gaits, walk, trot and canter, and are often homozygous for this nonsense mutation (AA). Four-gaited Icelandic horses can only perform tölt, in addition to walk, trot and canter and are often heterozygous (CA) for the mutation. Four-gaited Icelandic horses can also be homozygous for the wild-type gene (CC). In this study the significance of the *DMRT3*-mutation for how easy the Icelandic horse is to start tölt with has been tested. We also studied Standardbreds and Coldblooded trotters without the right genetic prerequisites to trot really fast on the racetracks to see if the mutation was significant for how easily they could be retrained to riding horses due to a better canter. The significance of the nonsense mutation has also been tested for the Morgan and American Curly to see if it has anything to do with how gaited the horses are. The study involved collection of hair samples and phenotypic description through questionnaires. In this study 263 hair samples have been analyzed. It has been shown that Icelandic horses homozygous for the wild-type gene are more difficult to put into tölt compared with horses that are hetero- or homozygous for the nonsense mutation. Standardbreds heterozygous for the *DMRT3*-mutation has been shown to be more easily retrained to riding horses due to a better canter compared to trotters that are homozygous for the nonsense mutation. The frequency of the *DMRT3*-mutation has been shown to have a large impact on how gaited the horse becomes in Morgans and American Curly.

1.0 INTRODUCTION

New research has identified a nonsense mutation in the *DMRT3*-gene at nucleotide position 22999655 on chromosome 23 that has a major impact on pattern of locomotion in horses (Andersson et al., 2012). During development the central pattern generation networks is created in the spinal cord and is responsible for the locomotor activity and the horses' pattern of movement (Grillner, 2006). The *DMRT3* is expressed in the neurons in the spinal cord and coordinates normal development of limb movement pattern. The nonsense mutation leads to a premature stop codon and a truncated *DMRT3*-protein. Equines without the nonsense mutation in *DMRT3* have the "wild"-type or naturally occurring gene (CC). Horses that are homozygous for the "wild"-type gene, (CC) can only perform the normally occurring gaits: walk, trot and canter/gallop. The *DMRT3*-mutation occurs with high frequency in horses that can perform alternative gaits, except for the naturally occurring gaits, walk, trot and canter. Alternative gaits can be pace, lateral ambling, diagonal ambling and regular rhythm ambling. The Icelandic Horse is an example of a gaited breed and they can, except for the naturally occurring gaits, also perform tölt and sometimes flying pace. Five-gaited Icelandic Horses can perform tölt and flying pace, except for the normally occurring gaits and are often homozygous (AA) for the nonsense mutation in *DMRT3*. Four-gaited Icelandic horses can perform tölt, except for the normally occurring gaits and are often heterozygous (CA) for the nonsense mutation, or homozygous for the "wild"-type gene (CC). The best performing trotters used for harness racing in Sweden can pace or trot at a very high speed without falling into their natural gait in high speed, gallop. These high performing Standardbreds are homozygous (AA) for the *DMRT3*-mutation (Andersson et al., 2012).

The breeding goal of gaits is not well defined for all breeds and today we don't know how large impact the *DMRT3*-mutation has on the gaits in the majority of different breeds. Equines have been bred for different purposes for example cross country, show jumping, dressage, alternative gaits or to trot really fast with a sulky. To match a horse with right conditions to a rider/driver is a prerequisite for a successful cooperation between equine and human. This is also important in an animal welfare perspective because if the horse owner knows the genetic prerequisites of the horse, the owner can match the horse with right purpose and not try to push the horse harder than what it capable of.

1.1 Specific aims

The Icelandic horse can sometimes be difficult to put into tölt and in this study it will be investigated if it has anything to do with the horse being a homozygous wild-type for the *DMRT3*-mutation or not. Available data of genotype frequencies from four-gaited Icelandic horses and random samples of Icelandic horses (Andersson et al., 2012) will be compared with the genotype frequencies for Icelandic horses in this study.

Trotters that don't have the right genetic prerequisites to trot really fast on the racetracks may have an alternative use in the riding sport instead. These horses may be better in canter and more easily retrained to riding horses. This will be investigated in both Standardbreds and Coldblooded trotters. There will be data made from not purposely selected Standardbreds were the genotype frequencies will be compared with the genotype frequencies for the Standardbreds collected to the study about riding qualities. Available random samples of genotype frequencies will also be compared with the genotype frequencies for Coldblooded Trotters in this study received from Lisa Andersson (unpublished data).

Today we don't know how the *DMRT3*-mutation is spread in the Morgan or in the American Curly and how it affects the gaits and that will be investigated in this study. Alternative gaits

in Morgan horses are desired by some people but others see it as a defect. There are some American Curly breeders in the world that breed for alternative gaits. This study is important because if it is possible to show that the ability to perform alternative gaits in these specific horse breeds is depending on the different genotype of *DMRT3* then horse owners can more easily choose horses that fit them.

The overall purpose with this study is to collect hair samples and phenotypic description of different breeds (Icelandic horse, Standardbred, Coldblooded trotter, Morgan and American Curly) and correlate the information with the horses´ genotype for *DMRT3*. The study will also include a survey of breeding goals for gaits of different horse breeds with different frequencies of the *DMRT3* mutation. Breeds included in the survey are; American Curly, American Paint, Campolina, Caspian, Coldblooded Trotter, Colombian Paso Fino, Finn horse, Icelandic horse, Kentucky Mountain Saddle Horse, Lewitzer, Mangalarga Marchador, Morgan, New Forest, Newfoundland pony, Orlov Trotter, Peneia, Pinos, Rocky Mountain Horse, Spanish Mustang, Standardbreds, Thessalion, Welsh Mountain and Welsh Pony.

1.2 Overview description of different gaits

For different breeds there are different definitions for different gaits. A short overview for the typical gaits will be described in this section.

Gaits can be defined by their support sequence, the pattern of the weight transfer when the horse is moving and also by the foot-fall timing and sequence. Gaits are divided into two categories; asymmetric- and symmetric gaits. The asymmetric gaits is when the horse is moving the legs differently from each other, like canter and gallop and the symmetric gait is when the movements on each side mirrors the movement on the other side, like trot.

Walk is a square gait, where the movement pattern is right hind-leg, right foreleg, left hind-leg and left foreleg, the gait is four-beat. Trot is a two-beat diagonal gait where the diagonal limbs for example left hind and right front lifts off and hits the ground at the same time. Pace is also a two-beat gait but with lateral footfall pattern where the lateral limbs moves forward and puts down at the same time.

There are different types of four-beat ambling gaits; diagonal ambling, lateral ambling and regular rhythm ambling where the hoof placement, timing and footfall pattern often are unique to different breeds (Andersson et al., 2012). Foxtrot is an example of diagonal ambling where the fore hooves of the horse hits the ground before the hind hooves. There are several different types of lateral ambling where the footfall timing and placement decides which type of lateral ambling the horse performs. An example for regular rhythm ambling is tölt where the footfall pattern is the same as for walk, but faster.

1.3 Breeding goals regarding gaits for the investigated horse breeds

Breeding goals of gaits are not well defined for all breeds and different horse breeds can perform several different gaits. Bellow follows a description of breeding goals for gaits for the breeds used in this study; the Icelandic horse, Standardbred, Coldblooded trotter, Morgan and American Curly. For more information about breeding goals for gaits in different breeds see Appendix 1.

1.3.1 Icelandic Horse

The Icelandic horse can perform one or two alternative gaits; tölt and sometimes flying pace in addition to walk, trot and canter/gallop. The breeding goals for gaits are well defined and the tölt should be four-beat, with lots of lift and action in the front legs with long strides in

both hind - and front legs. The movements should be very flexible and the horse should be able to perform tölt in different speeds. If the horse can perform flying pace it should be two-beat, lateral, very impressive with excellent suspension and good speed (Antonsson et al., 2011).

1.3.2 Swedish Standardbred

For the Swedish Standardbreds the goal is to breed for horses that have a clean trot and horses that are strong over distance. The Standardbred horse should have a winning instinct and be an outstanding trotter both on national and international level (ST, 2013).

1.3.3 Coldblooded Trotter

The breeding goal for Coldblooded Trotters is to develop and preserve a healthy competitive trotting horse. This is achieved by improving the trotters' competitive performance, conformation and temperament (ST, 2013).

1.3.4 Morgan Horse

In the breeding standard or in the ideal of the Morgan horse and in the judging standards for gait there is no alternative gaits described (AMHA, 2010; Lundin, 2010). However some breeders still breed for alternative gaits; fox trot, running walk, saddle rack and stepping pace. These gaits are common in gaited Morgans. The alternative gaits is said to go far back in time to the 17th century when Morgan horses were bred with horses that were able to perform alternative gaits (Burton, 2010).

1.3.5 American Curly

In the breeding standard for American Curly there are alternative gaits described; running walk and foxtrot, that some of the blood lines can perform (SACHF, 2004). In the Norwegian Curly horse Association there are five different groups of Curlies described; Classic Curly, Pleasure Curly, Western Stock Curly, Sport Curly and Draft Curly. It is most common that the Pleasure Curly is gaited (Norsk Curlyhestförening, 2013). There are breeders that breed on gaited Curlies that can perform hard pace, stepping pace, running walk, flat walk, rack/tölt, foxtrot and broken trot. Which gait the horse can perform depends on if it is more lateral or diagonal in its movement pattern (Jensens Curlyhester, 2013; pers.com. Jensen, 2013).

1.4 The role of the horse in the Swedish Society

The horse sector in Sweden is important because many people are involved in this sport in different ways. Two years ago the Swedish board of agriculture (2011) estimated the total number of horses to 362 700 in Sweden with 39 horses per 1 000 residents (SJV, 2011), with 35 different horse breeds and 16 different breeding associations (Braam, 2012). Riding sport is the third biggest sport among youths between 13-20 years of age (SCB, 2009). There are 33 racing tracks in the country (ST, 2012). About 1.5 hectares of land per horse is required when counting for pastures and feed production, this correspond to a total of 544 000 hectares of land (Braam, 2012). The horse sector creates 9 500 full-time employments (FTE) and additional employments of 9 000-18 000 FTE. About 10 billion SEK from a total of 20 billion SEK annual turnovers in the horse sector in Sweden are attributed to gambling. The horse sector contributes with 8 billion SEK to the GDP which correspond to 0.34 % of total GDP. From the gambling sector are 1.1 billion SEK tax revenues derived from a total of 4 billion SEK (Johansson et al., 2004). Since the horse sector in Sweden involves a lot of different people in the sport it would be of interest if this study could show that the factors that contribute to the horse ability to perform alternative gaits is depending on the different genotype for *DMRT3*. This because a rider/driver could be able to choose a horse more easily with the right prerequisites that fits them for a successful cooperation.

2.0 MATERIALS AND METHODS

In this study five different breeds were investigated to see how their gaits were affected by the *DMRT3*-gene; Icelandic horses, Standardbreds, Coldblooded trotters, Morgans and American Curly horses.

To find people that were interested in participating in this study information about the study was put online on different webpages; The Swedish Icelandic horse Association-SIF (www.icelandichorse.se), Svenska Ridtravarförbundet (www.ridtravare.com) and also in different representable groups at Facebook. Through a contact person at the Swedish Icelandic Horse Association, an email was sent out to 400 receivers. There were also phone calls made to horse owners and a large amount of emails were sent out. A notice was also put in a Swedish magazine called "Islandshästen". Several contact persons in different countries and in different breeding organizations were involved in spreading information about this study.

Before the study started, one of my supervisors; Lisa Andersson (Department of Animal Breeding and Genetics, SLU) put out information online on different webpages; the homepage of the Department of Animal Breeding and Genetics, at The Swedish University of Agricultural Science (SLU) (www.hgen.slu) and also on the Swedish Morgan Horse Association-SMHF (http://www.swedish-morganhorse.com). Articles and notices about the study was also published in two different Swedish magazines for Icelandic respectively trotting horses, called "Islandshästen" and "Travronden" before the study started.

2.1 Survey

Information about the frequency levels of the *DMRT3*-mutation in different horse breeds were received from Marta Promerová (Department of Medical Biochemistry and Microbiology, Uppsala University). Horse breeds with different frequency levels of the *DMRT3*-mutation were included in the survey. The purpose with the survey was to investigate if there were descriptions of breeding goals for gaits for different horse breeds that have different frequencies of the *DMRT3*-mutation and if there were anything described about alternative gaits for the different breeds. Also, if there were anything described about gaits that were not included in the breeding goals for gaits.

2.2 Questionnaires to horse owners

During autumn 2012 and early spring 2013, kits that consisted of questionnaires and/or material for taking hair-samples were sent to owners of Icelandic horses, Standardbred, Coldblooded trotters and Morgan. For more information about questionnaires see Appendix 2.

The American Curly were included from the survey and questionnaires have been received during spring 2013 (Appendix 2). A contact person for the American Curly put all information about kits on different webpages so that horse owners could print out the information them self.

Appendix 2 is written in English for Icelandic horses and American Curly, but the part for trotters are written in Swedish and not translated into English. The first part of the questionnaires consisted of questions about the owner and the horse, for example email address and registration number. The questions also contained several alternative answers to make it easier for the owners to answer the questions. Except for filling in the questionnaires, horse owners were also asked to collect hair samples from all above mentioned breeds and sent it in for analyzes. A reminder to answer the kits was sent out once to all breeds except for the American Curly. The collection of hair lasted until 1st of April.

Several contact persons in different countries have been involved in the collection of hair samples and questionnaires from different horse breeds others than those previously mentioned. There is an ongoing collection of samples from Colombian Paso Fino, Finn horses and Rocky Mountains that will not be analyzed in this study.

2.2.1 Icelandic horse

The criteria for participating in this study was for the Icelandic horses that they had to be difficult to be put into tölt and have four-gaited parents, but the criteria was modified later an and also horses that had five-gaited parents were included in the study. This modification was made to increase the number of horses.

Owners got questions about how many gaits the horses could perform, about the riding properties, how easy it was to start tölt the horse and also which scores they think the horse would got at a real or an imagined breeding evaluation. If the horse had not been on a real breeding evaluation the owners or trainers to the horse were asked to fill in the scores that they thought the horse would get on a breeding evaluation. Question 2 “which gait the horse prefers on pasture” and question 3 “which gait the horse picks under saddle before set to tölt” were included in the study to get a better knowledge about the horse. Information about parents for Icelandic horses was filled in afterwards from the online database WorldFengur.

2.2.2 Standardbred and Coldblooded Trotter

The criteria for participating were for trotters that they should be ridden. The questionnaire for Standardbreds and Coldblooded trotters was of the same type and contained questions about for how long time the trotter had been used as a riding horse, if it had competed in trotting races and also questions about the riding properties. The main questions was about how well the horse performed canter and how long time it had taken to get the horse to the canter ability it had today. Information about parents for the trotters were filled in afterwards from the online database; Swedish Trotting Association.

2.2.3 Morgan

The criteria for participating were for Morgans that the owners should be able to categorize their horses after number of gaits. Owners of Morgan horses did not receive any questionnaire but they were asked to categorize their horses after number of gaits, three-gaited (walk, trot and canter), four-gaited (walk, trot, canter and tölt) or five-gaited (walk, trot, canter, tölt and pace). A contact person collected this phenotypic description. The phenotypic information was then compared to the horses’ genotypes for *DMRT3*.

2.2.4 American Curly

For American Curly we focused on how gaited the horse was and this was also the criteria for participation, owners should be able to categorize how gaited their horses were. Except for the question “how gaited the horse was” in the questionnaire, horse owners were also asked to fill in information of which locomotion pattern the horse performed gaits in and what blood linage the horse had. There was also a question about if the horse had been ridden/driven when the gaits were observed or if the gaits had been observed without a rider/driver. We wanted to find out more about how gaited the horses were and match that with the horse genotype for *DMRT3*, because we do not know today how large effect the nonsense mutation in *DMRT3* have on the gaits for American Curly. Horses that only have the three normally occurring gaits; walk, trot and canter and none of the alternative gaits are referred to as not-gaited.

2.3 Isolation of DNA from hair roots

DNA was prepared from a standardized procedure. Hair samples were cut down into separate wells in a 96-well plate when prepared for analysis, with 4-5 hair sacks in each well. The solution was calculated to contain 100 µl Chelex (5%) and 7 µl proteinase K in each well. There was also one negative control. These samples were centrifuged for about one minute in order to mix the solution and hair sacks with each other. The 96-well plate was then put into a 56º C heat cabinet for one hour and after that the reaction was deactivated on a heat block for 10 minutes.

2.4 Genotyping

Before the samples could be analyzed in the StepOnePlus® Real-Time PCR System, (Life Technologies, 2013), samples were removed from the 96-well plate into a new 96-well plate with a total volume of 15 µl solution/well for one reaction. The solution consisted of 1.5 µl DNA, 7.5 µl Master Mix 2.0X, 0.38 µl Assay Mix 40X and 5.62 µl distilled water. Assays were performed according to manufacturer's instructions (Life Technologies, 2013).

Samples were genotyped using two markers, the nonsense mutation in *DMRT3* on nucleotide position 22999655 on chromosome 23 and 31999 bp upstream SNP BIEC2-620109, nucleotide position 22967656 on chromosome 23. Following primers were used: forward primer: CCTCTCCAGCCGCTCCT and reverse primer: TCAAAGATGTGCCCGTTGGA. Following probes were used: the wild-type probe: CTGCCGAAGTTCG and the mutant probe: CTCTGCCTAAGTTCG (Life Technologies, 2013).

The *DMRT3* marker was amplified in 50 cycles whilst the BIEC2-620109 was amplified in 40 cycles. Marker SNP BIEC2-620109 was used in order to double-check the result from marker *DMRT3_Ser301STOP* and to find possible recombinants.

The increase in fluorescence during PCR amplification occurs only when the cleavage by the DNA polymerase happens to probes that are hybridized to the target. Probes consist of a quencher dye at the 3′ end and in the 5′ end probes consist of a reporter dye. The cleavage occurs between the quencher dye and the reporter dye. During this cleavage the reporter dye gives the increased fluorescent signal and in intact probes that is suppressed. There is no non-specific amplifications detected (Life Technologies, 2010). The amplification of specific alleles gives the different genotypes of the horses.

Genotype frequencies for Icelandic horses in this study was compared with a group of random samples consisting of 162 Icelandic horses from Iceland and also a group consisting of 124 samples from evaluated four-gaited horses (Andersson et al., 2012).

Fifty-one Standardbred horses, mostly born in 2012, was not purposely selected and genotyped in order to be able to compare data of genotype frequencies with the data collected from the preselected Standardbreds for this study. DNA from the biobank was used from the laboratory at the Department of Animal Breeding and Genetics, at The Swedish University of Agricultural Science (SLU).

Random samples of genotype frequencies from 132 Coldblooded trotters received from Lisa Andersson (unpublished data) was also compared with the genotype frequencies for the Coldblooded trotters in this study.

2.5 Analysis of received samples for the different breeds

2.5.1 Icelandic Horse

From the total number of 171 Icelandic horses there were two groups made. Group "1" consisted of 84 horses including; horses with evaluated four-gaited parents, horses from non-evaluated parents and horses from parents with low pace scores (score <6.0). Group "2" consisted of all available Icelandic horses, both those included in the first group and also horses with highly evaluated five-gaited parents. In total included group 2, 151 horses. These 2 groups were made to be able to compare genotype frequencies and average scores from the questionnaire. Birth year for Icelandic horse in this study range from 1992-2009. Of all the genotyped samples for Icelandic horses were 5.8 % of the samples only genotyped with one marker, the *DMRT3_Ser301STOP* and not for both markers which were done for the rest, 94 %.

Horses excluded from further analysis were those that had incomplete answers on several questions and also those that had no answers on question number 5, "how easy it was to start tölt the horse". Some owners chose scores that included half scores on question 5 despite there were no such alternatives. These scores were included in the study. Some owners did choose several of the alternative answers on the questions about which gait the horse preferred on pasture (question number 2) and which gait it picked under saddle before set to tölt (question number 3). The answer was set as "something else" and not included in the statistics. If the owners wrote "something else" than trot, pace or neither on the pre-prepared answer on which gait the horse preferred when put into tölt (question number 4) the answers were corrected to neither and this data was not used in further calculations. If the owners had chosen to write "don't know" on the same question as before the answer was kept as don't know and not included in the statistics.

The last question (6); "which scores the horse would get on a real or imagined breeding evaluation", the alternative answers was either a whole or a half score. Several owners chose two alternatives, both a whole and a half score, and all of these have been corrected to the lower score. There were some owners that missed some answers at that question but they were still included in the results but not in the specific missed score. The scale for the scores for question number 6, range from 5-10 and score 5.0 indicated "gait not shown" and gait 10.0 was excellent.

2.5.2 Standardbred

All 44 received samples from Standardbred owners were included in the study. The Standardbreds in this study were born between 1985-2008, except for the not purposely selected sample were the Standardbreds were mostly born in 2012. In a few questionnaires some answers were incomplete, and those answers are not included in the study. There were a few owners that had chosen half scores for some questions despite there were no such answers, they were still included in the study. If the owners had chosen "don't know" on questions without alternative answers the results were not included in the statistics.

2.5.3 Coldblooded Trotter

All 24 received samples from Coldblooded trotter owners were included in the study. These questionnaires have been corrected in the same way as for the Standardbreds. Birth year for Coldblooded trotters in this study was 1989-2008, except for the random samples that were received from Lisa Andersson, were the trotters were born 1990-2010 (unpublished).

2.5.4 Morgan

All 34 received hair samples and phenotypic descriptions from Morgan horse owners were included in the study. The birth year for horses in this study was 1988-2011.

2.5.5 American Curly

There were 99 samples received from American Curly horse owners. Four samples were not used in the results; these included one horse with a Welsh pony mother, one horse that was a Missouri Fox trotter and two samples without phenotypic description of the gait. Horses in this study were born 1984-2012.

2.6 Analysis of data

Statistical analysis were made in Microsoft Excel, on the online webpage; Simple Interactive Statistical Analysis (SISA) (Quantitative Skills, 2013) and in the free software program for statistical computing, R (R Development Core Team, 2005).

For the Icelandic horses, Standardbreds and Coldblooded trotters Microsoft Excel was used for calculations of average, median, variance and standard deviation for the different genotypic groups for *DMRT3* and questions.

Fisher tests were made in the program R (R Development Core Team, 2005). For the Icelandic horses four questions (1-4) were analyzed; “is the horse four- or five-gaited”, “which gait the horse preferred on pasture”, “which gait the horse preferred before set to tölt” and also “which gait the horse preferred when set to tölt”. For Standardbreds four questions were analyzed (2, 7-9); if the horse had been competing in harness racings and/or dressage and also if the horse could perform tölt and/or pace. For Morgan and American Curly horses the Fisher test was used for the different genotypic groups for *DMRT3* and phenotypic groups.

For Icelandic horses and Standardbreds t-tests were made in SISA (Quantitative Skills, 2013) with a significance level of 0.05 and with a double sided hypothesis. If the p-value fell below the significance level the null hypothesis was rejected. For the Icelandic horses two questions were tested (5 and 6); “how easy it had been to start tölt the horse” and also “which scores the horse would get on a real or imagined breeding evaluation”. The scores for pace in question six were not included in the statistical calculations. For Standardbreds five questions were tested (3-6, 10); how well the horse could perform collected and extended canter, how well it performed collected and extended trot and walk. Also how difficult it had been to get the horse to the canter ability it has today and the horses jumping ability.

3.0 RESULTS

3.1 Answering frequency

For Icelandic horses, Standardbreds, Coldblooded Trotters and Morgans kits were received from Iceland, Norway and Sweden. For the American Curly kits were received from Canada, France, Germany, Norway, Sweden and USA.

Answering frequencies for the questionnaires sent out per horse were calculated (Table 1). For the Icelandic horses samples were also received from Lisa Andersson (Department of Animal Breeding and Genetics, SLU), Thorvaldur Arnason (IHBC), Freyja Imsland (IMBIM, UU) and her father Páll Þór Imsland. Lisa Andersson collected 26 samples before the study started and genotyped 19 of them and 25 samples were received from Lisa Andersson and Thorvaldur Arnason that they collected during their visit to Iceland. Freyja Imsland and her father Páll Þór Imsland collected 89 samples from Iceland that Freyja had already genotyped.

The questionnaires received from Freyja Imsland and her father Páll Þór Imsland has been translated into English from Icelandic.

The answering frequency for the American Curly could not be calculated due to that a contact person put all information about kits on different online webpages so that horse owners could print out the information them self.

Table 1. Answering frequency for kits for the different breeds in this study

Breed	No of kit sent out	No of kits received	Answering freq.
Icelandic horse	33	31(+26+25+89)*	0.94
Standardbred	64	44	0.69
Coldblooded Trotter	46	24	0.57
Morgan	42	34	0.81
American Curly	?	99	?

*Lisa Andersson collected 26 samples before the study started and she and Thorvaldur Arnason collected 25 samples from Iceland. Freyja Imsland and her father Páll Þór Imsland collected 89 samples from Iceland.

3.2 Genotyping

A total of 263 samples were analyzed and four of them failed during genotyping and got undetermined. Table 2 summarizes the genotype and allele frequency for the different horse breeds in this study. The table includes all material received in this study, random samples from Icelandic horses, the evaluated four-gaited Icelandic horses and the random samples from the Coldblooded trotters.

Table 2. Genotype and allele frequencies for DMRT3 for the horse breeds included in this study, with specific reasons for inclusion

			Genotype freq.			Allele freq.	
Breeds	Reason for study	No	AA	CA	CC	A	C
Icelandic Horse (group 1)[1]	Set to tölt	84	0.25	0.60	0.15	0.55	0.45
Icelandic Horse (group 2)[1]	Set to tölt	151	0.36	0.54	0.09	0.64	0.36
Icelandic Horse[2]	Four-gaited	124	0.31	0.67	0.02	0.64	0.36
Icelandic Horse[2]	Random sample	162	0.78	0.22	0.00	0.89	0.11
Standardbred	Riding properties	44	0.89	0.11	0.00	0.94	0.06
Standardbred	Random sample	51	1.00	0.00	0.00	1.00	0.00
Coldblooded Trotter	Riding properties	23	0.17	0.44	0.39	0.39	0.61
Coldblooded Trotter[3]	Random sample	132	0.11	0.54	0.36	0.38	0.63
Morgan	Gaitedness	34	0.09	0.29	0.62	0.24	0.76
American Curly	Gaitedness	93	0.56	0.29	0.15	0.70	0.30

[1]Group 1- horses with evaluated four-gaited parents, horses from non-evaluated parents and horses from parents with low pace scores (score <6.0)
[1]Group 2- consisted of all available Icelandic horses, both those included in the first group but also horses with highly evaluated five-gaited parents
[2]Evaluated four-gaited Icelandic Horses and randomly selected Icelandic horses (Andersson et al., 2012).
[3]Random selection of Coldblooded Trotters, Lisa Andersson (unpublished data).

3.2.1 Icelandic Horse

One sample failed twice during genotyping and was not included in the study. The genotype frequencies differed to some extent between the preselected groups. Group 1 had little higher frequency of genotype CC 15 % vs. 9 % and lower frequency of genotype AA 25 % vs. 36 %. The frequency of the wild-type gene, CC, was much higher in both the preselected groups comparing to the random samples from Iceland and the group with only evaluated four-gaited

horses (Andersson et al., 2012). For those groups the frequency of CC was 0 % and 2 % respectively (Table 2). The highest frequency of allele A (89 %) was in the random sample (Andersson et al., 2012) whiles the highest frequency of allele C (45 %) was discovered in group 1 with strictest preselection. Otherwise almost the same conclusions could be drawn from both group 1 and 2 and therefore only results from the group with more horses, group 2, were chosen to be shown in this study.

The genotype frequencies for the preselected group in relation to whether the horse was categorized as four- or five-gaited are shown in Table 3. The frequency of five-gaited horses was 15 % and two of the five-gaited horses had the genotype CA instead for AA.

Table 3. DMRT3 genotype frequencies for four-and five-gaited Icelandic horses

		Genotype freq.		
	Number	AA	CA	CC
4-gaited	129	0.27	0.62	0.11
5-gaited	22	0.91	0.09	0.00

3.2.2 Standardbred

The frequency of CA in Standardbreds was much higher in the group of horses used as riding horses compared to the not purposely selected horses (11 % vs. 0 %) (Table 2). Four recombinants (CT/AA) were detected giving a recombination frequency of 9.1 %.

3.2.3 Coldblooded Trotter

There was one sample that failed twice during genotyping and was not included in the study. One Coldblooded trotter was recombinant between the two markers (CT/CC) giving a recombinant frequency of 4.3 %. For the trotters preselected based on riding properties the frequency of AA and CC was higher with 17 % and 39 % compared to the data from the random sample with 11 % and 36 % (Table 2). The allele frequencies for the preselected group and the Coldblooded trotters in the random sample were almost the same, allele A 39 % vs. 38 % and allele C 61 % vs. 63 %.

3.2.4 Morgan

Through the genotyping there was one recombinant discovered in the Morgans, between the two markers (TT/CA) giving a recombinant frequency of 2.9 %. Horses had been divided into three different phenotypic groups depending on how many gaits the horse could perform and the result is summarized in Table 2. Of the 23 horses with three gaits, 83 % were CC, of the six four-gaited horses 83 % were CA and of five five-gaited horses 60 % were AA (Table 4).

Table 4. DMRT3 genotype frequencies for the different phenotypes in Morgans

		Genotype freq.		
Phenotype	No	AA	CA	CC
Three-gaited	23	0.00	0.17	0.83
Four-gaited	6	0.00	0.83	0.17
Five-gaited	5	0.60	0.20	0.20

3.2.5 American Curly

For the American Curly there were two samples that failed during analyzes, these are not included in the study. In this breed horses were divided into four different phenotypic groups, depending on how gaited the horse was. The genotype frequency for each gait category is shown in Table 5. Of the 26 not-gaited horses (or three-gaited) 35 % were CC, of 12

somewhat gaited horses 50 % were CA, of 24 gaited horses 79 % were AA and of 31 strongly gaited horses 94 % were AA.

Table 5. DMRT3 genotype frequencies for the different phenotypes in American Curly

		Genotype freq.		
Phenotype	No	AA	CA	CC
Not-gaited	26	0.00	0.65	0.35
Somewhat Gaited	12	0.33	0.50	0.17
Gaited	24	0.79	0.17	0.04
Strongly Gaited	31	0.94	0.00	0.06

3.3 Correlation between gaits and genotypes

Descriptional data have been calculated for Icelandic horses and Standardbreds and is presented in more details in Appendix 3, supplementary information (Table 1-3).

3.3.1 Icelandic Horses

Almost the same conclusions could be drawn from both preselected groups and therefore was the group with more horses chosen to be shown (group 2). Significant results that differ will be mentioned.

There is a significant correlation between genotype and gaitedness in Icelandic horses ($p \leq 0.0001$) which is shown in Table 6. Four-gaited horses were most often CA and five-gaited horses were most often AA. Horses were not included in the calculations in Table 6 if owners picked answers that were not in the pre-prepared answers. The pre-prepared answers are not shown for questions were the answering frequency was zero.

Table 6. Number of horses with different genotypes for different results from the questionnaire, and significance level from Fisher test for the effect of different genotypes of DMRT3 on gait properties for Icelandic horses

Question	Alternatives	No of horses AA	CA	CC	Significance level
1. Is the horse four- or five gaited?	Four-gaited	35	80	14	
	Five-gaited	20	2	0	
					p≤0.0001
2. Which gait does the horse prefer on pasture, running at medium speed? [1]	Trot	41	82	14	
	Tölt	2	0	0	
	Piggy-pace	1	0	0	
					n.s.*
3.Wich gait did the horse pick under saddle before set to tölt [2, 3]	Trot	40	78	14	
	Tölt	8	0	0	
	Piggy-pace	3	1	0	
					p≤0.001
4.Did the horse go towards trot or pace when set to tölt [4,5,6]	Trot	8	40	10	
	Pace	22	13	1	
					p≤0.0001

*n.s. means that the result was not significant.
[1] 11 horses with AA not included in the Fisher calculations due to another gait or a mixture of gaits.
[2] 4 horses with AA not included in the Fisher calculations due to another gait or a mixture of gaits.
[3] 3 horses with CA not included in the Fisher calculations due to another gait or a mixture of gaits.
[4] 25 horses with AA not included in the Fisher calculations due to another gait or a mixture of gaits.
[5] 29 horses with CA not included in the Fisher calculations due to another gait or a mixture of gaits.
[6] 3 horses with CC not included in the Fisher calculations due to another gait or a mixture of gaits.

For question number 2; “which gait the horse preferred on pasture when running at medium speed”, there were no significant results. For question number 3; “which gait the horse preferred under saddle before set to tölt”, it was affected by the genotype (p≤0.001). Horses that were AA went more towards tölt than horses that where CA or CC, these horses went more towards trot except for one CA that went towards pace before set to tölt. For question number 4; “if the horse went towards trot or pace when set to tölt” was significantly affected by the genotype (p≤0.0001). Horses with AA in genotype went more towards pace than trot compared to CA and CC where most of the horses went towards trot. This question was not significant for group 1.

Table 7 shows the averages for question 5 and 6 for the different genotypes for *DMRT3*. Icelandic horses with an AA in genotype had the lowest average for question number 5; “how easy it was to start tölt your horse” and horses with a CC in genotype had the highest scores. Also when put together the genotypes AA and CA they had still a lower average score (3.49) for how easy it was to start tölt compared with horses that had CC in genotype. Horses with AA in genotype had also the highest scores for tölt at a real or an imagined breeding evaluation (question number 6). Horses with CC in genotype had the lowest scores for all gaits at a real or imagined breeding evaluation.

Table 7. The average scores for the different DMRT3 genotypes for Icelandic horses

		Genotype		
		AA	CA	CC
Question	Alternatives	Average	Average	Average
5. How easy was it to start tölt your horse (score 1-6)[1]	5. Put into tölt	2.36	4.26	5.25
6. Which score do you think your horse would get at a breeding evaluation (score 5-10)[2]	6. Walk	7.82[3]	7.89[4]	7.53
	6. Trot	8.08	8.21	7.79
	6. Tölt	8.81	7.62[5]	6.08[6]
	6. Pace	6.12	5.06[5]	5.00
	6. Canter	8.32	8.23[5]	7.64

[1]Score 1 was very easy.
[2]Score 5 indicates gait not shown.
[3]54 horses instead of 55 in the statistical calculations due to no answer.
[4] 80 horses instead of 82 in the statistical calculations due to no answer.
[5] 81 horses instead of 82 in the statistical calculations due to no answer.
[6] 13 horses instead of 14 in the statistical calculations due to no answer.

For question 5; "how easy was it to start tölt your horse", Icelandic horses with AA was easier to put into tölt compared to both CA ($p \leq 0.0001$) and CC ($p \leq 0.0001$) as seen in Table 8. Horses with CA were also easier to put into tölt compared to horses with CC ($p \leq 0.01$). Also when put together the different genotypic groups AA and CA they were easier ($p \leq 0.0001$) to put into tölt compared to horses with CC in genotype.

For question 6; "which scores would your horse get at a breeding evaluation", there were no significant difference for walk when comparing the average assessment scores for the different genotypic groups for *DMRT3*. Icelandic horses with a CA in genotype had higher average assessment scores for trot compared to horses with CC ($p \leq 0.05$). But there was no significant difference for average assessment scores for trot when comparing horses with AA and CA in genotype or when comparing AA with CC. For group 1, horses with AA in genotype had higher average assessment scores ($p \leq 0.05$) for trot comparing to horses with CC in genotype, this was not significant for group 2.

Icelandic horses with AA had higher average assessment scores for tölt at a real or an imagined breeding evaluation compared to both CA ($p \leq 0.005$) and CC horses ($p \leq 0.0001$). Also horses with CA in genotype had significant higher scores for tölt at a real or a possible breeding evaluation compared to horses with CC in genotype ($p \leq 0.005$). When the groups of AA and CA horses were put together they had still higher average assessment scores for tölt compared to horses with CC ($p \leq 0.0001$).

It has also been shown for both horses with AA and CA in genotype to have significant higher average assessment scores for canter at a real or an imagined breeding evaluation compared CC-horses, ($p \leq 0.005$ and $p \leq 0.01$). There were no significances detected for the different genotypic groups in average assessment scores for walk.

Table 8. Significance levels from T-tests for differences in average assessment scores between horses with different DMRT3-genotypes in Icelandic horses

		Genotype		
		AA-CC	CA-CC	AA-CA
		Significance	Significance	Significance
Question	Alternatives	level	level	level
5. How easy was it to start tölt your horse (score 1-6)	5. Put into tölt	$p \leq 0.0001$	$p \leq 0.01$	$p \leq 0.0001$
6. Which score do you think your horse would get at a breeding evaluation (score 5-10)	6. Walk	$p > 0.05$	$p > 0.05$	$p > 0.05$
	6. Trot	$p > 0.05$	$p \leq 0.05$	$p > 0.05$
	6. Tölt	$p \leq 0.0001$	$p \leq 0.005$	$p \leq 0.005$
	6. Canter	$p \leq 0.005$	$p \leq 0.01$	$p > 0.05$

3.3.2 Standardbreds

In Table 9 the results from the Fisher test for the different genotypes of *DMRT3* and performance data are presented. There was no significant difference between the two different genotypes; AA and CA for question number 2; "if the horse had been competing in harness racings before retrained to a riding horse". For question number 7 and 8 there were no significant differences for both genotypic groups if the horse could perform tölt or pace. Also for question number 9; "if the horse had been competing in dressage" there were no significant differences in answers between the two different genotypes; AA and CA.

Table 9. Number of horses with different genotypes among horses with different results from the questionnaire, and significance levels from Fisher test for the different genotypes of DMRT3 and performance data in Standardbreds

		No of horses		Significance
Question	Alternatives	AA	CA	level
2. Been competing in harness racings before retrained to a riding horse[1]	Yes	28	3	
	No	10	2	
				n.s.
7. Can your horse perform tölt[2]	Yes	8	0	
	No	28	5	
				n.s.
8. Can your horse perform pace[3]	Yes	15	0	
	No	21	5	
				n.s.
9. Have your horse ever been competing in dressage	Yes	12	3	
	No	27	2	
				n.s.

[1]1 horse with AA in genotype not included in the Fisher calculations due to the owner did not know if the horse had performed in harness racings.

[2] 3 horses with AA in genotype not included in the Fisher calculations due to the owners did not know if their horses could perform tölt.

[3]3 horses with AA in genotype not included in the Fisher calculations due to the owners did not know if their horses could perform pace.

Table 10 summarizes number of Standardbreds for the different genotypes of *DMRT3*, average scores and also t-tests relating to riding properties. Trotters with AA in genotype had the lowest average scores for the different gaits and jumping ability.

Table 10. Results from T-test for average assessment scores relating to riding properties compared for the different genotypes for DMRT3 in Standardbreds

Question	Alternatives* (score 1-6)	Genotype AA Average	Genotype CA Average	Significance level
3a. How well does your horse perform collected canter	3a. Rhytm	3.99	5.10	$p \leq 0.025$
	3a. Balance	3.90	5.00	$p \leq 0.025$
	3a. Transitions	3.44	4.80	$p \leq 0.05$
3b. How well does your horse perform extended canter	3b. Rhytm	4.26	5.00	n.s.
	3b. Balance	4.05	5.00	$p \leq 0.05$
	3b. Transitions	3.64	4.80	$p \leq 0.025$
4. Hard to get the canter ability it has today	4. Canter ability	2.92[1]	3.00	n.s.
5a. How well does your horse perform collected trot	5a. Rhytm	4.38[1]	5.20	n.s.
	5a. Balance	4.36[1]	5.20	n.s.
	5a. Transitions	3.91	5.00	$p \leq 0.025$
5b. How well does your horse perform extended trot	5b. Rhytm	4.18[1]	4.40	n.s.
	5b. Balance	4.11[1]	4.40	n.s.
	5b. Transitions	3.80	4.00	n.s.
6. How well does your horse perform Walk	6. Rhytm	4.85	5.20	n.s.
	6. Balance	4.85	5.60	$p \leq 0.05$
	6. Transitions	4.60	5.20	n.s.
10. How are the horses jumping ability	10. Jumping ability	4.33[2]	5.25[3]	n.s.

*Score 1 is bad and 6 is excellent for all questions except for question 4 where 1 is easy and 6 is difficult.
[1] 38 horses instead of 39 in the statistical calculations due to no answers.
[2] 36 horses instead of 39 in the statistical calculations due to no answers.
[3] 4 horses instead of 5 in the statistical calculations due to no answer.

When comparing the average scores for question number 3a and b, trotters with a CA in genotype performed collected and extended canter better than trotters with AA in genotype. This was significant for rhythm, balance and transitions in question 3a ($p \leq 0.025$, $p \leq 0.025$ and $p \leq 0.05$), for question 3b it was only significant for balance and transitions ($p \leq 0.05$ and $p \leq 0.025$). There was no significant difference between the two genotypic groups in question four; "how hard it has been to get horse to the canter ability it has today".

For question number 5a trotters with CA in genotype had it easier with the transitions in collected trot ($p \leq 0.025$) and with the balance in walk for question number 6 ($p \leq 0.05$). Rhythm and balance in question 5a (collected trot) was not significant and rhythm and transitions was not significant for question number 6 (walk). Also there were no significant differences in average scores between the two genotypic groups; AA and CA in extended trot (question 5b) or the jumping ability (question 10).

3.3.3 Coldblooded Trotters

In the supplementary information in Appendix 3 (Table 4) are different average scores for riding properties shown for Coldblooded trotters. There were too few individuals to calculate any significance. The number of horses with answers in questions 2 and 7-9 is presented in Table 11. Many of the horses in this study had been competing in harness before participating in this study (question number 2). Trotters with AA in genotype could perform both tölt and pace while none of the horses with CC could perform tölt and only one of them could perform pace (question number 7 and 8). Almost none of the Coldblooded trotters in this study had ever been competing in dressage (question number 9).

Table 11. Number of Coldblooded trotters in question 2 and 7-9 and their DMRT3 genotype

		No of horses		
Question	Alternatives	AA	CA	CC
2. Been competing in harness racings before retrained to a riding horse	Question 2			
	Yes	2	7	4
	No	2	3	5
7. Can your horse perform tölt[1]	Question 7			
	Yes	4	2	0
	No	0	8	7
8. Can your horse perform pace	Question 8			
	Yes	3	0	1
	No	1	10	8
9. Have your horse ever been competing in dressage[2, 3]	Question 9			
	Yes	0	1	1
	No	4	7	7

[1] 2 horses with CC not included due to the owners didn't know if the horse could perform tölt.
[2] 2 horses with CA not included due to the owners didn't know if the horse had been competing in dressage.
[3] 1 horse with CC not included due to the owner didn't know if the horse had been competing in dressage.

The average score for Coldblooded trotters for collected and extended canter was almost the same for all three different genotypes, although the average for CC was slightly lower. The average score for how difficult it had been to get the horse to the canter ability it has today was relatively low on the six-point scale. CC horses had a little higher average score in collected trot compared to the other genotypes. Trotters with a CA in genotype had the lowest average for extended trot. The highest average for walk and jumping ability was for horses with CC in genotype.

3.3.4 Morgan

There was a significant correlation between genotypes for *DMRT3* and gaitedness in Morgan horses ($p \leq 0.0001$) Three-gaited horses were most often CC, four-gaited horses CA and five-gaited horses AA (Table 12).

Table 12. Number of horses with different genotypes among those with different gaits and significance level from Fisher test for the different genotypes of DMRT3 and different gaits in Morgan

	No of horses			Significance
Phenotype	AA	CA	CC	level
Three-gaited	0	4	19	
Four-gaited	0	5	1	
Five-gaited	3	1	1	
				$p \leq 0.0001$

3.3.5 American Curly

There was a significant correlation between genotypes for *DMRT3* and gaitedness in American Curly ($p \leq 0.0001$). Not-gaited horses that only perform walk, trot and canter were most often CA, somewhat-gaited horses CA, gaited horses were most often AA and strongly gaited horses were also most often AA (Table 13).

Table 13. Number of horses with different genotypes among those with different gaitedness and significance level from Fisher test for the different genotypes of DMRT3 and gaitedness in American Curly

	No of horses			Significance
Phenotype	AA	CA	CC	level
Not-gaited	0	17	9	
Somewhat Gaited	4	6	2	
Gaited	19	4	1	
Strongly Gaited	29	0	2	
				$p \leq 0.0001$

4.0 DISCUSSION

4.1 Icelandic Horse

There were two five-gaited Icelandic horses with a CA in genotype in this study, the frequency was 9 %. This may be due to that the owners are very skilled riders. The reason could also be a wrong phenotypic description, maybe these horses were very lateral in their movements in fast tölt and therefore the owners think they were five-gaited. Experimental errors do happen sometimes, for example there could have got something wrong with the genotyping; switched samples or an allelic dropout. One of the Icelandic horses that had a CC in its genotype had a sire that had received 7.5 points for pace at a breeding evaluation. There were also another Icelandic horse with CC in genotype and this horse had a dam that had received score 6 for pace. Another example was a horse that had a CA in genotype but both the dam and the sire were evaluated for being five-gaited and had received 7 points for pace. Perhaps this depend on other genes that we don't know of today or maybe the rider to these horses that had received scores for pace were excellent good in getting the horse in flying pace. For future studies it could be interesting to investigate horses with CA in genotype which had been scored as five-gaited in greater detail.

A lot of horses with the right genetic prerequisites for being five-gaited may still be evaluated for being four-gaited, this because pace is a demanding gait. There are several factors that affect the horse ability to perform pace and some of those are: the rider, the age of the horse and also the temperament of the horse. A huge factor for getting the horse in pace is if the horse has received properly training before putting into pace.

When comparing the genotype frequencies for group 1 and 2 the frequency of CC was higher in group 1 with 15 % vs. 9 %. This corresponds with our thoughts that the frequency of CC is higher in certain groups of horses. When comparing the material from only evaluated four-gaited Icelandic horses (Andersson et al., 2012) with both the preselected group in this study the frequency of genotype CC was higher in the preselected groups. One reason for why the frequency of CC was lower in the group with only evaluated four-gaited horses (2 %) (Andersson, 2012) could be that Icelandic horses that are CC participates more seldom in breeding evaluations because of more difficulties to show the different gaits. Probably these horses need even more skilled riders than what is usual. The frequency of CC in the random sample for Icelandic Horses on Iceland was zero. Maybe Icelanders are more careful not to breed two four-gaited horses because of the risk for getting a four-gaited horse that is hard to put into tölt, which also supports our hypothesis in this study. Horses that were CC in this study were the hardest ones to start tölt when compared to horses with AA and CA, while horses with AA in genotype were the easiest ones to put into tölt. For question number 5;" how easy was it to start tölt your horse", several owners had picked scores that included half intervals. This may probably be due to that on question number 6; "which scores do you think

your horse would get at a breeding evaluation", owners could pick scores that included half intervals. For future studies should half intervals be included also for question number 5.

It has been seen through the Fisher test that horses for question number 4; "did the horse go towards trot or pace when set to tölt", was depending on which genotype they had. Horses with CA and CC went more towards trot than pace, while horses with AA went more towards pace. This was not significant for group 1 which could depend on that there were fewer horses in the calculation. Several owners had chosen alternatives for this question that were not included in the pre-prepared answers. This question should be rewritten for future studies with more alternative answers.

Icelandic horses with CC had the lowest average assessment scores for all gaits at a real or an imagined breeding evaluation. This was also significant for trot for group 1 when comparing AA with CC and CA with CC but for group 2 this was only significant when comparing CA with CC. It was also significant for horses with CC that they had lower average assessment scores for canter but not for walk. Maybe the preselection for this study collected the most difficult Icelandic horses with CC and CA in genotype, because there were also significant differences between the horses with AA and CA in genotype when they were put into tölt. But here we should keep in mind that not all horses were evaluated at breeding evaluations and maybe the owners underestimated their horses' capacity.

4.2 Standardbred

Out of the 44 Standardbred samples only five horses had the genotype CA. This was although much higher compared to the random samples where the number was zero. The higher frequency of CA in this study could depend on the preselection of horses that were used as riding horses. In the Fisher test there were no significances detected and this could be because of too few participants that were heterozygous for the *DMRT3*-mutation. None of the horses with CA in genotype was performing tölt or pace while nine of the horses with AA in genotype performed tölt and 15 performed pace. If more samples would be collected from horses preselected for riding qualities there would probably be significant results in the Fisher test for the ability to perform tölt and/or pace.

The Standardbreds in this study that were CA performed collected and extended canter better than trotters with AA in genotype which also matched with the hypothesis. But for the question about how difficult it had been to get the horse to the canter ability it had today the answer was not significant. This could depend on too few samples with the genotype CA. Trotters with CA in genotype seem to have it easier with the transitions in collected trot and with the balance in walk. For future studies it would be interesting to continue the collection of samples and try to find more horses that are CA in genotype or maybe even CC because then probably there would be more and higher significances among the results.

4.3 Coldblooded Trotter

For Coldblooded trotters there were only 24 received samples and these were too few to be able to calculate any significances. It was interesting that horses with AA in genotype performed both tölt and pace in much higher frequency than horses with CA and CC in genotype, and only one CC could perform pace. This follows the same pattern as for the Standardbreds in this study. When comparing the horses in this study with the random sample of 132 horses received from Lisa Andersson (unpublished data) there were not so big differences in the frequencies. Maybe there are no such big differences between genotypes when comparing the ability for the horses to become riding horses. The collection of more

samples from Coldblooded trotters should continue in the future so that significances can be calculated.

The average score for collected and extended canter was almost the same for all three different genotypes, although the average for CC was slightly lower. The average score for how difficult it had been to get the horse to the canter ability it has today was relatively low on the six-point scale. This could depend on that many of the horses didn't have a well-balanced canter and the question was misunderstood. The question should be rewritten for future studies. Horses with CC had a little higher average score in collected trot compared to the other genotypes. Trotters with a CA in genotype had the lowest average for extended trot The highest average for walk and jumping ability was for horses with CC in genotype.

4.4 Morgan

Of the 34 samples for Morgan horses did 79 % of the samples match with the genotype and phenotype and also with the hypothesis for this study. Four of the three-gaited horses were CA in genotype and one reason for this could be that the owners had not been able to detect any alternative gaits. One of these horses was only three years old and the alternative gaits may appear later in life. That CA or AA horses scores as a three-gaited is thus not that surprising. However, one of the four-gaited Morgan horses was a CC in genotype. This horse was 22 years old and in addition there were also two five-gaited Morgans where the genotype and phenotype did not match. One of these horses was a CA in genotype and seven years old. This horse had been seen to perform both tölt and pace under a rider and the mother to this horse was four-gaited and there was a clear difference between the performance of gaits for mother and offspring. The mother had not ever been seen to perform pace. The second five-gaited Morgan were the genotype and phenotype did not match was 19 years old and a CC in genotype. This horse had been seen to perform pace under a rider but never tölt, so this horse was clearly a gaited Morgan. Also here there could have been an experimental error for example there could have got something wrong with the genotyping; switched samples or an allelic dropout.

In America the alternative gaits for gaited Morgans are described as running walk, fox trot, saddle rack and stepping pace (Burton, 2010). Maybe there are other genes involved in the pattern of locomotion that we do not know of today. To be able to find a pattern and draw some final conclusions for horses that do not have a matching phenotype and genotype the collection of gaited Morgans should go further an. To this study there were too few gaited Morgans received compared to the not-gaited horses that only could perform walk, trot and canter.

4.5 American Curly

In this study in total had 95 % of the horses a matching genotype and phenotype that also matched with the hypothesis for this study. In the not-gaited group (horses that only can perform walk, trot and canter) there were 17 horses with CA in genotype, these horses were born between 1997-2012. Some of these horses could have a wrong phenotypic description. The low age could affect the phenotype, alternative gaits can come later an in life. Also if the owner does not know that the horse has alternative gaits maybe the horse is never being put into these either. Two of the 12 somewhat gaited American Curly horses in this study were CC in genotype. The first horse could instead of a "normal" trot perform a type of foxtrot during riding. The second horse was thought to be somewhat gaited. One of the 24 gaited horses was a CC in genotype and this horse had sometimes been seen to move in an ambling gait. Sometimes a horse that feels pain for example in the back or in a leg can move in a special way to unburden the pain and be categorized as gaited or be believed to have

alternative gaits. Two of the 31 horses that were strongly gaited had a CC in their genotype. One of these horses had been seen to perform ambling and pace. The second horse could perform running walk. It could also here bee other genes involved in the pattern of locomotion.

5.0 CONCLUSION

Icelandic horses with a CC in genotype were significantly more difficult to put into tölt compared with horses that where AA or CA in genotype. These horses had also lower scores for tölt, trot and canter. Standardbreds that were heterozygous for the DMRT3-mutation had better canter ability with a rider compared to the trotters that were homozygous for the DMRT3-mutation. For Morgan and American Curly it has been shown through this study that the ability to perform alternative gaits is highly depending on their DMRT3 genotype.

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APPENDIX

Appendix 1

Survey-Breeding goals with focus on alternative gaits in horses

American Paint

There was nothing found in the breed information about gaits or alternative gaits (APHA, 2013; SPHA, 2012).

American Saddlebred

The American Saddlebred can perform alternative gaits, slow-gait and a faster "rack". The breed should always be able to move in these extra gaits because it is in the breeds properties (Nordic American Saddlebred Society, 2012). Slow-gait is a highly collected, slow four-beat gait were the lateral front- and hind hoof starts in the same time but the hind hoof hits the ground slightly before the lateral fore hoof (ASHA, 2012b) characterized of high and airy movements with a lot of attitude (ASHA, 2012a). The gait is from the beginning developed from pace (ASHA, 2012a). Rack is a faster form of the slow gait (ASHA, 2012b; pers.com. Larsson, 2012) and if the horse shows any signs of pace or trot when performing rack it will get a lower score on competitions (ASHA, 2012a). There are Saddlebreds that perform pace but that is not desirable (pers.com.Andersson, 2012; pers.com. Larsson, 2012).

Campolina

In the breeding standards the perfect gait for the Campolina is *Marcha de Centro* an even lateral and diagonal gait, which is performed as a middle point between trot and pace (Four Beat Gaited Horse, 2013). There have been different breeds used in the Campolina and until year 1934 the Mangalarga Marchador was used in the breed to refine and give better proportions to the horse. Campolina also inherited the extra four-beat gaits, *Marcha batida* and *Marcha picada* from Mangalarga Marchador (Hendricks, 1995).

Caspian

In the International Caspian Society there are only breeding standards described for walk, trot and canter/gallop (The International Caspian Society, 1965).

Colombian Paso Fino

The Paso Fino is born with a unique, natural, very soft four-beat lateral gait where the hoofs are moved in a regular, independently sequence in exact intervals. The movement pattern is started with right hind, right front, left hind and left front, the hind limb hits the ground slightly before the front limb. The gait is performed in three different speeds (presented in increasing speed): Classic Fino, Paso Corto and Largo Corto (PFHA, 2012). But there are also some horses that are born with a more diagonal gait, the Trocha which is a four-beat uneven diagonal gait similar to the Foxtrot (pers. com. Blichmann, 2013). Only about 30 % of all Paso Finos can perform the Classic Fino which is performed with minimal extension and highly collection, all gaited Paso Finos can do the Corto and Largo, some rare horses can also only perform the Fino. In Colombia they only have shows for Classic Fino disciplines which mean that the Paso Finos are only performing one gait. In the United States there are several different shows that Classic Fino horses can compete in but probably with not so good results. There are different breeding goals for horses when breeding for different show disciplines. Horses in Colombia that can't perform the Classic Fino are often sold as working horses. The Paso Finos in Colombia were many years ago crossed with a trot horse and some offspring would perform the Trocha and some would perform the Paso Fino gait. There are also horses that do not perform these extra gaits that are characteristic for this breed (pers.com. Blichmann, 2013).

Finn horse

Performance result is the main criteria for breeding, all horses should have three pure gaits and the trotters must be able to trot really fast and have a well-balanced trot (Hendricks, 1995; Suomenhevonen, 2007), but some of them can also pace, which is forbidden (Suomen Hippos, 2012). The breed of Finn horse is divided into four breeding categories; working-, riding-, trotting- and small horse, but the breed is universal and all Finn horses can be used in all different branches (Suomenhevonen, 2007).

Kentucky Mountain Saddle Horse

In the breed standard it is stated that the Kentucky Mountain Saddle Horse should perform a single foot gait that is four-beat, natural and very smooth (KMSHA, 2012). In the Kentucky Mountain Saddle Horse Association (KMSHA) (2012) Show Catalog and Rulebook there are a "show" gait and a pleasure gait described. The pleasure gait is a saddle rack that is performed with more speed than the show gait.

Lewitzer

Nothing could be found of breeding goal for gaits. There are three categories of Lewitzer, category one is the smallest with the same wither height as the Shetland pony. Category two is used for working on the fields and category three is used for larger children (Hendricks, 1995).

Mangalarga Marchador

The breed has two extra four-beat gaits; *Marcha Batida* and *Marcha Picada* where the *picada* is the smoothest (Hendricks, 1995; Guerra, 1997). In the *picada* the legs move more in a lateral pattern than in a diagonal (USMMA, 2012), like a broken pace with trippel hoof support in periods (Hendricks, 1995; Guerra, 1997). *Batida* is when the horse moves the legs more in a diagonal pattern than in a lateral (USMMA, 2012) and it is like a broken trot with trippel hoof support and the fore hoof is overreached by the hind hoof on the same side (Hendricks, 1995; Guerra, 1997). In the recent years there have also been also a third gait discovered but that is still not recognized as a distinct own gait, it is called *Marcha de Centro* and is an even lateral and diagonal gait (USMMA, 2012). *Marcha Picada* and *Marcha Batida* are judged separately in shows (Four Beat Gaited Horse, 2013).

New Forest

There is nothing said about alternative gaits in the breeding standard, only that the movements should be free, straight and active without being exaggerated (The New Forest Pony Breeding Society & Cattle Society, 2011; Svenska New Forest Föreningen, 2012). The New Forest Pony is used for riding in many different disciplines and also for lighter draft work (Hendricks, 1995).

Newfoundland pony

There is no breeding goal for gaits or alternative gaits in the Newfoundland pony but some of the ponies can perform tölt and there are also some breeders that want to preserve this alternative gait. Tölt is believed to be inherited from the extinct Galloway Pony (pers.com. Mehaney, 2012).

Orlov Trotter

Nothing could be found of breeding goal for alternative gaits in this breed.

Peneia

This breed can perform an extra gait which is a sort of pace, called *aravani* (Hendricks, 1995). The breeding goals for gaits could not be found for this breed.

Pinos

The breeding goals for gaits could not be found for this breed.

Rocky Mountain Horse

In the bylaws there is an extra gait described that is lateral and smooth ambling with an equal rhythm of four distinct hoof beats which can be performed in different speeds (RMHA, 2012). In the Rocky Mountain Horse Associations (RMHA) Show Rules and Regulations (2011) there are show walk and a Rocky Mountain pleasure gait described. The pleasure gait is a lateral four-beat gait with more speed than in the show walk. There are still some horses that can perform the pleasure gait diagonally, but then the question is if these horses can be accepted as Rocky Mountain horses. There are also horses that are hard to put into the pleasure (pers.com. Swan and Swan, 2013).

Spanish Mustang

All gaits are acceptable in this breed as long as it is smooth and rhythmic, every individual will perform an extra gait on its own individual way and different movement pattern can be presented (SMR, 2008). Nothing more could be found of gait description.

Thessalian

The breeding goal for gaits could not be found for this breed.

Welsh Mountain

In the breeding goal for gaits it is stated that the action of the ponies should be free, quick, straight and powerful but there is nothing written about alternative gaits (The Welsh Pony & Cob Society, 2000).

Welsh Pony

The breeding goal for gaits is the same as for the Welsh Mountain Pony (The Welsh Pony & Cob Society, 2000). But nothing could be found for if horses sometimes could move in another way than walk, trot and canter.

Appendix 2

Gaits in Icelandic horses

Name of trainer:……

Name of horse:……..

Registration number (type carefully):……..…….

Date, stable and city:……

Please circle the answer that best fit your horse

1. Is the horse five-gaited or four gaited?

four five don't know yet

2. Which gait does the horse pick when running at medium speed in the pasture?

trot toelt piggypace slow canter

3. Before set to toelt, which gait did the horse pick under saddle?

trot toelt piggypace slow canter

4. Did the horse go towards trot or pace when set to toelt?

trot pace neither

5. How easy was it to start toelt on your horse (1= very easy)?

1 2 3 4 5 6

6. Which score do you think your horse would get at a breeding evaluation (circle or make a cross between the numbers if x.5)?

Walk:	5	6	7	8	9	10
Trot:	5	6	7	8	9	10
Toelt:	5	6	7	8	9	10
Pace:	5	6	7	8	9	10
Canter:	5	6	7	8	9	10

Thank you!

Studie om travhästars ridbarhet

Hästens namn: ……..…

Hästens IDnummer: ……..…

Ras: ……..…

Hästägare/stad: ……..…

Email adress: ……..…

Telefonnummer: ……..…

Var vänlig och svara kortfattat på frågorna nedan alternativt ringa in rätt svar.

1. Hur många år har hästen använts som ridhäst?

Vet ej

2. Hade hästen startat som travhäst innan den skolades om till ridhäst?

Ja Nej Vet ej

3a. Hur väl behärskar din häst samlad galopp (1= dålig, 6= utmärkt)?

Takt	1	2	3	4	5	6
Balans	1	2	3	4	5	6
Övergångar	1	2	3	4	5	6

Kommentar:

3b. Hur väl behärskar din häst ökad galopp (1= dålig, 6= utmärkt)?

Takt	1	2	3	4	5	6
Balans	1	2	3	4	5	6
Övergångar	1	2	3	4	5	6

Kommentar:

4. Hur svårt det har varit att få hästen till den galoppförmågan som den har i dag (1= lätt, 6= svårt)?

1 2 3 4 5 6

Kommentar *(ex har hästen tränats regelbundet i dressy x år?):*

5a. Hur är din häst i samlad trav (1= dålig, 6= utmärkt)?

Takt	1	2	3	4	5	6
Balans	1	2	3	4	5	6
Övergångar	1	2	3	4	5	6

Kommentar:

5b. Hur är din häst i ökad trav (1= dålig, 6= utmärkt)?

Takt	1	2	3	4	5	6
Balans	1	2	3	4	5	6
Övergångar	1	2	3	4	5	6

Kommentar:

6. Hur är din häst i skritt (1= dålig, 6= utmärkt).

Takt	1	2	3	4	5	6
Balans	1	2	3	4	5	6
Övergångar	1	2	3	4	5	6

Kommentar:

7. Går din häst någonsin i tölt *(även väldigt långsam räknas)*?

Ja Nej

8. Går din häst någonsin i pass *(även väldigt långsam räknas)*?

Ja Nej

9a. Har hästen någonsin tävlat i dressyr?

Ja Nej
Vet ej

9b. Om ja, vilken är den högsta klassen den ställt upp i?

9c. Om nej, varför?

10. Hur är hästens hoppförmåga (1= dålig, 6= utmärkt)?

1 2 3 4 5 6

Kommentar:

11. Övriga kommentarer:

Gaits in the American Curly Horse

A genetic study by the University of Agricultural Sciences in Uppsala, Sweden

The university will analyze hair samples from at least 20 Curly Horses from each of the categories non gaited, diagonally gaited, square gaited and laterally gaited. The results form the base for a master study of student Niina Kangas. The purpose of the study is to look at the DMRT3 gene and the locomotion in different breeds.

Horse owner:……..

Email: ..... Telephone: .....

Name of horse:……

Year of Birth: ..... Gender: .....

Registration number(s) (type carefully):……..……...

Sire: …….…..…….

Reg.number(s): ……..

Dam: …….…….

Reg.number(s): ……..

Is your horse:

☐ Not gaited ☐ Somewhat gaited ☐ Gaited

☐ Strongly gaited ☐ Don't know

When you look at the pedigree of your horse, how much gaited blood does it have? (circle one)

0% 25% 50% 75% 100% Don't know

If your horse falls between two options, choose the one that matches best with the abilities that have been passed on to next generation. For instance if 5 of 8 great grandparents are gaited = 62.5% gaited blood. But if the one grandparent that is of 50% gaited blood is not gaited, then go down to 50% for your horse. Or if that grandparent is gaited, go up to 75% for your horse.

If your horse has gaited blood in its pedigree, which linage(s) does the gait come from? (if there are more than one major line, circle all those)

☐ Foxtrotter (Walker's Prince T / Sir Patrick MJT / Star's Lucky Touch or foxtrotters in pedigree)

☐ Mustang

☐ Damele line

☐ Other Curly blood line

☐ Other non curly gaited breeds

If your horse is gaited, please answer to your best ability

The horse's natural gaits are mostly:

☐ Diagonal (flat walk, foxtrot, broken trot etc)

☐ Square (flat walk, running walk, rack/tölt)

☐ Lateral (stepping pace, hard pace)

☐ I don't know

Does your horse also trot?

☐ Yes, often ☐ Yes, but rarely ☐ Never or almost never ☐ Don't know

The gaits of the horse have been observed from:

☐ Paddock or in hand (movements without rider) ☐ Movements when ridden/driven

Has your horse been included in the Ancestral DNA study of ICHO:

☐ Yes ☐ No ☐ Don't know

I agree that the hair sample from my horse is used in the study “Gaits in the American Curly Horse” and agree to that the results may be published.

Place..... Date.....

Signature .....

Thank you! When the study is completed a report will be available to you by internet or email. Questions? Email Niina Kangas at nika0002@stud.slu

Appendix 3

Supplementary information

Table 1. Descriptional data for the different genotypes of DMRT3 for Icelandic Horses

		No of horses			AA	CA	CC
Question	Alternatives	AA	CA	CC	Proportion	Proportion	Proportion
1. Is the horse four-	1. Four-gaited	35	80	14	0.64	0.98	1.00
or five-gaited	1. Five –gaited	20	2	0	0.36	0.02	0.00
2. Which gait does the	2. Trot	41	82	14	0.75	1.00	1.00
horse prefer on pasture,	2. Tölt	2	0	0	0.36	0.00	0.00
running at medium	2. Piggy-pace	1	0	0	0.18	0.00	0.00
speed	2. Slow canter	0	0	0	0.00	0.00	0.00
	2. Something else	11	0	0	0.20	0.00	0.00
3. Which gait did the horse	3. Trot	40	78	14	0.72	0.95	1.00
pick under saddle before	3. Toelt	8	0	0	0.15	0.00	0.00
set to tölt	3. Piggy-pace	3	1	0	0.05	0.01	0.00
	3. Slow Canter	0	0	0	0.00	0.00	0.00
	3. Something else	4	3	0	0.07	0.04	0.00
4. Did the horse go towards	4. Trot	8	40	10	0.15	0.49	0.71
trot or pace when set to	4. Pace	22	13	1	0.40	0.16	0.07
tölt	4. Neither	25	21	3	0.45	0.26	0.21
	4. Don't know	0	8	0	0.00	0.01	0.00

Table 2. Descriptional data for the different genotypes of DMRT3 for Icelandic horses in this study

		No of horses			AA	CA	CC	AA	CA	CC	AA	CA	CC
Questions	Alternatives	AA	CA	CC	Median	Median	Median	Variance	Variance	Variance	Stdev.	Stdev.	Stdev.
5. How easy was it to start tölt your horse (score 1-6)	5. Set into tölt	55	82	14	2.00	4.50	5.25	1.61	1.63	0.45	1.27	1.28	0.67
6. Which scores do you think your horse would get at a breeding evaluation (score 5-10)	6. Walk	54[1]	80[2]	14	8.00	8.00	8.00	0.30	0.44	0.83	0.55	0.66	0.91
	6. Trot	55	82	14	8.00	8.50	8.00	0.71	0.38	0.30	0.84	0.62	0.54
	6. Tölt	55	81[3]	13[4]	8.50	8.00	5.50	0.77	1.17	1.66	0.88	1.08	1.29
	6. Pace	55	81[5]	14	5.00	5.00	5.00	2.09	0.13	0.00	1.44	0.35	0.00
	6. Canter	55	81[6]	14	8.50	8.00	8.00	0.43	0.39	0.44	0.65	0.62	0.66

[1] 1 horse not included in the calculations due to no scores for walk.

[2] 2 horses not included in the calculations due to no scores for walk.

[3,4]1 horse respectively not included in the calculations due to no scores for tölt.

[5,6] 1 horse respectively not included in the calculations due to no scores for walk.

Table 3. Descriptional data for Standardbreds relating to riding properties

Question	Alternatives	No of horses AA	No of horses CA	AA Median	CA Median	AA Variance	CA Variance	AA Stdev.	CA Stdev.
3a. How well does your horse perform	3a. Rate	39	5	4.00	5.00	1.66	0.55	1.29	0.74
collected canter	3a. Balance	39	5	4.00	5.00	2.09	0.50	1.44	0.71
	3a. Transitions	39	5	4.00	5.00	2.00	0.20	1.41	0.45
3b. How well does your horse perform	3b. Rate	39	5	4.00	5.00	1.67	0.51	1.29	0.71
extended canter	3b. Balance	39	5	4.00	5.00	1.89	0.50	1.38	0.71
	3b. Transitions	39	5	4.00	5.00	1.92	0.70	1.39	0.84
4. Hard to get the canter ability it has today	4. Canter ability	38[1]	5	3.00	3.00	1.53	1.50	1.24	1.22
5a. How well does your horse perform	5a. Rate	38[1]	5	5.00	5.00	1.13	0.70	1.06	0.84
collected trot	5a. Balance	38[1]	5	4.00	5.00	1.07	0.70	1.03	0.84
	5a. Transitions	39	5	4.00	5.00	1.24	0.50	1.11	0.71
5b. How well does your horse perform	5b. Rate	38[1]	5	4.00	4.00	1.94	1.30	1.39	1.14
extended trot	5b. Balance	38[1]	5	4.00	4.00	1.39	1.30	1.18	1.14
	5b. Transitions	39	5	4.00	4.00	1.87	0.50	1.37	0.71
6. How well does your horse perform	6. Rate	39	5	5.00	5.00	1.77	0.20	1.33	0.45
Walk	6. Balance	39	5	5.00	6.00	1.50	0.30	1.23	0.55
	6. Transitions	39	5	5.00	5.00	1.84	0.20	1.35	0.45
10. How are the horses jumping ability	10. Jumping ability	36[2]	4[3]	4.33	5.25	5.00	5.50	1.71	0.92

[1] 1 horse not included in the calculations due to no answers for the different questions.
[2] 3 horses not included in the calculations due to no answer.
[3] 1 horse not included in the calculations due to no answers.

Table 4. Averages scores for different genotypes of DMRT3 for Coldblooded trotters in this study

		Genotypes		
		AA	CA	CC
Question	Alternatives*	Average	Average	Average
3a. How well does your horse perform collected canter	3a. Rate	3.75	4.10	3.56
	3a. Balance	4.00	3.90	3.33
	3a. Transitions	4.00	3.50	2.67
3b. How well does your horse perform extended canter	3b. Rate	5.33[1]	4.90	4.67
	3b. Balance	5.33[1]	4.80	4.22
	3b. Transitions	4.67[1]	4.22	2.89
4. Hard to get the canter ability it has today	4. Canter ability	2.00	3.00	2.70
5a. How well does your horse perform collected trot	5a. Rate	4.50	4.70	5.33
	5a. Balance	4.50	4.40	4.78
	5a. Transitions	3.50	4.30	4.89
5b. How well does your horse perform extended trot	5b. Rate	5.00	4.40	4.89
	5b. Balance	5.00	4.30	4.89
	5b. Transitions	4.00	3.60	4.89
6. How well does your horse perform Walk	6. Rate	5.00	5.00	5.78
	6. Balance	5.00	5.10	5.56
	6. Transitions	4.50	4.70	5.33
10. How are the horses jumping ability	10. Jumping ability	3.50[2]	3.83[3]	4.67

*Score 1 is bad and 6 is excellent for all questions except for question 4 where 1 is easy and 6 is difficult.
[1] 3 horses instead of 4 in the average calculation due to no answers.
[2] 2 horses instead of 4 in the average calculation due to no answers.
[3] 6 horses instead of 10 in the average calculation due to answers.