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Riding Ability in Icelandic Horses
– Effect of Conformation and the ‘Gait Keeper’ Mutation in the *DMRT3* Gene

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Clarification of contribution

I hereby declare that the writing of the following thesis and the three accompanying papers is my work, done with supervision of Dr. Sigríður Björnsdóttir, Dr. Þorvaldur Árnason and Dr. Ágúst Sigurðsson. Dr. Nathalie Crevier-Denoix and Dr. Philippe Pourcelot provided the 3-D video morphometric method used for the quantification of conformation and supervised its application.

The contribution of Þorvaldur Kristjánsson to the papers included in the thesis was as follows:

Paper I: Kristjánsson collected all data which are presented in the paper, performed quantification of the conformation of studied horses and performed the statistical analyses. All co-authors contributed to the interpretation of the results. Kristjánsson drafted the paper, further revised by the other co-authors. Kristjánsson was responsible for correspondence with the scientific journal.

Paper II: Kristjánsson collected all data which are presented in the paper and performed the statistical analyses. Klonowski performed the genotyping of horses. Árnason performed the genotype probability estimations. Kristjánsson interpreted the results together with the co-authors. Kristjánsson drafted the manuscript, further revised by the co-authors. Kristjánsson was responsible for correspondence with the scientific journal.

Paper III: Kristjánsson collected all data which are presented in the paper, performed quantification of the conformation of studied horses and performed the statistical analyses. Albertsdóttir performed the estimation of genetic parameters. Kristjánsson interpreted the results together with the co-authors. Kristjánsson drafted the manuscript, further revised by the co-authors. Kristjánsson was responsible for correspondence with the scientific journal.

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Þorvaldur Kristjánsson

Abstract

Kristjánsson, T. 2014. Riding ability in Icelandic horses - Effect of conformation and the 'Gait keeper' mutation in the *DMRT3* gene. Doctoral thesis

The breeding goal for the Icelandic horse promotes a five-gaited riding horse with a functional and aesthetically appealing conformation. The main objective of the thesis was to estimate the association between conformation and riding ability in Icelandic breeding horses. The first step was to apply and evaluate in terms of repeatability a three-dimensional (3-D) video morphometric method for quantification of the conformation. The second step was to assess the effect of the *DMRT3* 'Gait keeper' mutation on gaiting ability in the Icelandic horse and estimate the development in frequency of the mutation within the population. The third and final step was to assess the association between conformation and riding ability, where both standard (direct) and 3-D conformational measurements were related to breeding field test scores for riding ability.

The conformation of 72 breeding horses was quantified with the 3-D morphometric method, which provided objective, detailed and repeatable data on the conformation and was found to be suitable for description of the breed and further studies. Comparison with earlier studies confirmed that the Icelandic horse has grown taller in recent years, changed from a rectangular to square body format and acquired a more uphill conformation. Measurements of the joint angles of the limbs revealed carpal and tarsal valgus and fetlock valgus to be frequent findings in the breed.

The second part of the thesis involved genotyping of 667 breeding horses with respect to the *DMRT3* 'Gait keeper' mutation. The majority (76.3%) was homozygous mutant (AA) and 22.5% were heterozygous (CA) while only 1.2% was found homozygous for the wild type allele (CC). Homozygosity for the 'Gait keeper' mutation was confirmed to be permissive for the ability to pace and had a favourable effect on scores for the lateral gait *tölt*, demonstrated by better beat quality, speed capacity and suppleness. Horses that were heterozygote for the mutation had, on the other hand, significantly higher scores for the basic gaits and performed better beat and suspension in trot and gallop. These results indicate that the AA genotype reinforces the coordination of ipsilateral limbs, with the subsequent negative effect on the synchronized movement of diagonal limbs compared with the CA genotype. Change in the frequency of the mutation was estimated on the basis of genotype probabilities of 146,763 horses registered in WorldFengur. The frequency of the A-allele has increased in the population in recent decades with a corresponding decrease in the frequency of the C-allele, most likely promoted by the emphasis on lateral gaits in the breeding goal. The estimated frequency of the A-allele in the Icelandic horse population in 2012 was 0.94 with a predicted loss of the C-allele in relatively few years.

The final step was to estimate the phenotypic and genetic relationship between standard conformational measurements and scores for the riding ability and determine if 3-D morphometric measurements could discriminate between high-class and low-class horses based on scores for the different gaits. The data comprised records from all assessed breeding horses in Iceland in 2000-2013 (10,091 horses) and a subpopulation of 98 haphazardly selected breeding horses with a detailed quantification of the conformation in 3-D. Most of the standard measurements had a significant curvilinear relationship with the studied riding ability traits. Different 3-D measurements could discriminate between high-class and low-class horses within each gait with high accuracy by multivariate analyses, as well as between AA horses that were presented as four-gaited horses (without pace) and five gaited horses with good pacing ability. Proportions in the top line of the horse describing the height of the horse at front compared to hind were found to be most important for the riding ability, revealing the advantage of an uphill conformation. Their estimated heritability and genetic correlation with total score for riding ability designate them as important indicators for performance.

The results have practical implications for breeding of Icelandic horses and can improve the assessment of the conformation at the breeding field tests and consequently the riding ability of the Icelandic horse.

Keywords: 3-D objective quantification, conformation, gaiting ability, *DMRT3* genotype effect, genotype probability, standard conformational measurements

Ágrip

Þorvaldur Kristjánsson 2014. Ganghæfni íslenskra hrossa - Áhrif sköpulags og breytileika í *DMRT3* erfðavísinum

Opinbert ræktunarmarkmið íslenska hestsins hvetur til ræktunar úrvals reiðhesta með fimm gangtegundir og sköpulag sem stuðlar að ganghæfni, heilbrigði og fegurð. Aðalmarkmið þessa verkefnis var að meta samband sköpulags og ganghæfni hjá íslenska hestinum. Fyrsti hlutinn fólst í mælingu á líkamsbyggingu hrossa með þrívíðri myndbandsgreiningu. Í öðrum hluta voru áhrif breytileika í *DMRT3* erfðavísinum á ganghæfni íslenskra hrossa metin og þróun í tíðni A og C samsætanna áætluð. Þriðji og síðasti hlutinn fólst í mati á sambandi sköpulags og ganghæfni, þar sem kannað var samband hefðbundinna skrokkmála og nákvæmari mælinga á líkamsbyggingu hrossa við einkunnir fyrir hæfileika í kynbótadómum.

Í fyrsta hluta var líkamsbygging (s.s. lengdir beina, hæð frá jörðu og horn liða) 72 kynbótahrossa mæld með þrívíðri myndbandsgreiningu sem skilaði hlutlægum og nákvæmum upplýsingum, að jafnaði með háu tvímælingargildi og þótti aðferðin henta til frekari rannsókna. Samanburður við eldri rannsóknir sýndi að íslenski hesturinn hefur hækkað á undanförnum árum, einkum að framan, en á sama tíma orðið hlutfallslega styttri. Fram kom að kiðfætt/kýrfætt fótstaða og útskeif staða um kjúkur á bæði fram- og afturfótum eru algeng frávik frá beinni fótstöðu.

Í öðrum hluta rannsóknarinnar var arfgerð 667 kynbótahrossa greind með tilliti til breytileika í *DMRT3* erfðavísinum. Meirihluti þeirra (76,3%) reyndist arfhreinn fyrir stökkbreyttu samsætunni (AA), 22,5% voru arfblendin (CA) og 1,2% voru arfhrein fyrir stofngerðar samsætunni (CC). Það var staðfest að AA arfgerðin er skilyrði fyrir skeiðgetu og hafði ennfremur jákvæð áhrif á einkunnir fyrir tölt, sem skýrist að hluta af meiri mýkt og rými hjá AA hrossum. Hross sem voru arfblendin höfðu aftur á móti hlotið hærri einkunnir fyrir grunn gangtegundirnar og voru að jafnaði takthreinni og svifmeiri á brokki og stökki. Þessar niðurstöður bentu til þess að AA arfgerðin styrki samhæfingu hliðstæðra fóta og hafi neikvæð áhrif á samstilltar hreyfingar skástæðra fóta. Þróun í tíðni samsætanna var metin á grunni upplýsinga um líkur á *DMRT3* arfgerð 146.763 hrossa sem eru skráð í gagnagrunninn WorldFeng. Tíðni A samsætunnar hefur aukist í stofninum síðustu áratugi á kostnað C samsætunnar. Ætla má að áhersla á tölt og skeið í ræktunarmarkmiðinu hafi stuðlað að þessari breytingu. Áætluð tíðni A samsætunnar í íslenska hrossastofninum árið 2012 var 0,94 og haldi fram sem horfir mun C samsætan hverfa úr stofninum innan fárra ára.

Í síðasta hluta rannsóknarinnar var lagt mat á svipfars- og erfðafylgni á milli hefðbundinna skrokkmála og einkunna fyrir hæfileika. Gögnin innihéldu alla dóma á Íslandi frá 2000 til 2013 (10.091 hross). Einnig var kannað hvort unnt væri að greina á milli úrvalshrossa og lakari hrossa á grundvelli nákvæmari líkamsmála 98 hrossa sem fengin voru með þrívíddarmælingum og var þá litið til hverrar gangtegundar fyrir sig. Hin hefðbundnu skrokkmál höfðu flest marktækt boglínulagað samband við einkunnir fyrir hæfileika. Líkamsmál sem fengin voru með þrívíddarmælingum gátu greint á milli úrvalshrossa og lakari hrossa af miklu öryggi þegar fjölþátta tölfræðigreiningu var beitt. Þau gátu einnig greint á milli arfhreinna AA hrossa sem voru sýnd sem fjórgangshross annars vegar og fimmgangshrossa með úrvals skeiðgetu hins vegar. Hlutföll í yfirlínu hestsins sem lýsa hæð hans að framan miðað við að aftan höfðu áhrif á allar gangtegundir og bentu til þess að háar herðar, bein baklína og hátt settur háls hafi jákvæð áhrif á ganghæfni. Arfgengi hlutfalla í yfirlínunni sem tengjast háum herðum og erfðafylgni þeirra við aðaleinkunn hæfileika styðja þá niðurstöðu.

Niðurstöður verkefnisins hafa hagnýta þýðingu fyrir ræktun íslenska hestsins og geta bætt mat á sköpulagi hrossa á kynbótasýningum, þar sem sköpulagsþættir sem stuðla að eðlisgóðri ganghæfni fá aukið vægi.

Lykilorð: Þrívíð myndbandsgreining, sköpulag, ganghæfni, skrokkmál, *DMRT3* erfðavísir

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List of Original Papers

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I. Kristjansson, T., Björnsdóttir, S., Sigurðsson, A., Crevier-Denoix, N., Pourcelot, P. Árnason, T. 2013. Objective quantification of conformation of the Icelandic horse based on 3-D video morphometric measurements. *Livestock Science* 158, 12-23.

II. Kristjánsson, T., Björnsdóttir, S., Sigurðsson, A., Andersson, L.S., Lindgren, G., Helyar, S.J., Klonowski, A.M., Árnason, T. 2014. The effect of the ´Gait keeper´ mutation in the *DMRT3* gene on gaiting ability in Icelandic horses. *Journal of Animal breeding and Genetics* DOI: 10.1111/jbg.12112.

III. Kristjansson, T., Björnsdóttir, S., Albertsdóttir, E., Sigurdsson, A., Pourcelot, P., Crevier-Denoix, N., Árnason, T. Association of conformation and riding ability in Icelandic horses. Submitted for publication in *Livestock Science*.

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Publication II is reprinted with kind permission of *Journal of Animal Breeding and Genetics.*

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1 Introduction

The ultimate goal in horse breeding is to produce horses with a functional and durable conformation combined with great performance. Many horse breeds worldwide are bred for multiple purposes where selection is based on various traits of conformation, performance and behavior (Koenen *et al.*, 2004). The Icelandic horse is a multi-gaited horse breed suited for leisure riding and sport competitions (FEIF, 2014). The main focus in its breeding is consequently on the riding ability together with functional and aesthetically appealing body conformation. The international breeding goal for the Icelandic horse is extensive and incorporates 15 traits of conformation and riding ability.

The relationship between conformation and performance has been the subject of a number of studies which have shown that various conformational traits are favorable to performance in different disciplines (Holmström *et al.*, 1990; Back *et al.*, 1996; Crevier-Denoix *et al.*, 2006; Weller *et al.*, 2006b). In order to estimate the relationship between conformation and performance it is important to have objective, repeatable and accurate quantification of both the conformation and performance (van Weeren & Crevier-Denoix, 2006). The assessment of conformation of the Icelandic horse is currently based on the subjective scoring of composite traits, selected by empirical evidence and experience. Incorporating scientific knowledge about its relationship with riding ability is a key issue for the development of the breed. It was therefore of great interest to objectively quantify the conformation of the Icelandic horse and relate to data on riding ability, which is produced annually at breeding field tests.

A nonsense mutation in the *DMRT3* gene (*DMRT3_Ser301STOP),* also referred to as the ´Gait keeper´ mutation, has been shown to have a great impact on gaiting ability in horses and DMRT3 is reported to be crucial for the normal development of a coordinated locomotor network that controls limb movement (Andersson *et al.,* 2012). Detailed estimation of the effect of the mutation on gait quality in the Icelandic horse was therefore necessary before the relationship between conformation and riding ability could be assessed.

1.1 Background information

The Icelandic horse is the only horse breed in Iceland, where it has developed since the settlement during the period 874-930. The breed is considered pure-bred as the import of foreign genetic material has been insignificant from that period (Adalsteinsson, 1981). The horses which founded the breed originated mainly from Norway and the British Isles

(Bjørnstad & Røed, 2001). The population size has fluctuated in the past mainly due to weather conditions and volcanic eruptions. The first counting of horses in Iceland revealed 26,910 horses in 1703 and in 1784 they had reduced to about 8,600 as a consequence of the Laki volcanic eruption (Bjarnason, 1966; Icelandic Statistics, 1997). Today, the population size exceeds 75,000 horses in Iceland and 250,000 worldwide (www.Worldfengur.com). The Icelandic horse was the only means of transportation on land until the last century and had a role in traditional agricultural work. The use of the Icelandic horse as a draught horse was never extensive and it was predominantly used for riding and as a pack horse. Because of late industrialization the horse kept its importance well into the 20th century. Today the Icelandic horse is mainly bred for leisure riding and special gait competitions and is a popular riding horse in Iceland as well as in 30 countries of Western Europe and North America. Because of extensive export of breeding horses from the Icelandic population, the genetic relationship between horses is almost the same within a country as across countries (Árnason & Sigurðsson, 2004; Hreiðarsdóttir *et al.*, 2014). The history of systematic selection of the Icelandic horse is not long as selection based on defined breeding goals began in the middle of the 20th century. Therefore, a considerable phenotypic variation is found within the breed for conformation, color and performance.

One of the defining characteristics of horse breeds is their ability to perform alternate gaits. A gait is a characteristic coordination pattern of the limbs identified by the timing and sequence of the footfalls. The gait chosen by a horse depends on speed, genotype and environmental factors such as type of training (Alexander, 1988; Clayton, 2004). The Icelandic horse is a multi-gaited horse showing the standard gaits of all domestic horse breeds, i.e. walk, trot, canter and gallop. In addition it has *tölt* and pace. *Tölt* is a four-beat running gait with lateral sequence of footfalls and without suspension. Pace is a lateral gait with a moment of suspension where lateral limbs move almost synchronously back and forth and is optimally a very fast gait (Clayton, 2004). Icelandic horses that have the ability to perform walk, trot, canter/gallop and *tölt* are referred to as four-gaited horses, whereas horses that additionally have the ability to perform pace are five-gaited horses.

The breeding system for the Icelandic horse comprises a definition of the breeding goal, registration of horses, subjective assessment of horses at breeding field tests, and estimation of breeding values. Breeding values have been evaluated with the use of a multi-trait BLUP animal model since 1983 (Árnason, 1984b), and from 2004 the evaluation has been performed for Icelandic horses in 12 countries. The estimated heritability of conformation and riding ability traits in Icelandic horses have been reported to range from

0.22 to 0.46 and from 0.20 to 0.63, respectively, and estimated genetic correlation between conformation and riding ability traits from -0.24 to 0.54, with most traits having a positive correlation (Albertsdóttir *et al.*, 2008). Considerable genetic gain according to the breeding goal has been achieved over the last decades (Árnason & Sigurðsson, 2004; Sigurðardóttir, 2011) and, although the native Icelandic horse population is a closed population, the level of genetic diversity is still acceptable with the effective population exceeding 100 horses (Kristjánsson, 2005; Hreiðarsdóttir *et al.*, 2014).

1.2 Breeding goal

The studbook of the Icelandic horse breed was established in 1923 and the first breeding field test was organized by the Agricultural Society of Iceland in 1906. A subjective scoring system for the evaluation of the breeding horses was first elaborated in 1950. The first description of the breeding goal for conformation is most likely based to some extent on the Danish guidelines *Vejledning i Bedømmelsen af Hestens Ydre* [Instructions in assessment of the conformation of horses] by Harald Goldschimdt, first published in 1892. The scoring index has been subject to minor and major modifications since 1950, with respect to number of traits and their weight in the total score. The last modification to the scoring index was made in 2010. The international breeding goal for the Icelandic horse describes a versatile five-gaited horse with a functional and elegant conformation, a manageable temperament and willingness to perform. The horse should be robust; healthy, fertile and durable and all color variations are to be maintained within the breed. The breeding goal allows for considerable variation in size, but a preferred range in height is 135 cm to 145 cm when measured with a stick. The scoring index comprises 15 subjectively scored composite traits, which fit the specific definition of the breeding goal and are the main criteria for selection. These are eight conformation traits and seven performance traits, which have a 40% and 60% weight in the total score, respectively (FEIF, 2014).

The conformation should be aesthetically appealing and enhance natural gaiting ability. The horse should be light-built, with an emphasis on flexibility, strength and muscularity. The head should be finely built, with fine and well positioned ears. The neck withers and shoulders are combined in one trait: the neck should promote a good head carriage and be long and fine, high set and well raised, the withers should be high and long and the shoulder should be sloping and long. The back and croup are assessed as one trait and should be well muscled. The back should be broad and its lowest point should be in the middle of the back; the croup should be long, sloping and evenly formed. As for proportions the horse should be well-proportioned, with the front part, midsection and hind quarters of equal length; it should be higher at withers than at croup, rectangular, with long limbs and a light, cylindrical body. For the assessment of limbs and hooves emphasis is on strength and a correct limb stance from the lateral view; the forelimbs should be straight from elbow to the pastern with an intermediate slope (ca 50°) as well as length of the pasterns and the hind limbs should not be camped out (the point of buttock and hock should be in an vertical line when standing squarely). The correctness of the limbs is also judged in the frontal and rear view; the front limbs should be straight with adequate space between them. The same applies for the hind limbs, although they may turn out slightly. The horse should have abundant mane and tail. The Icelandic horse should be able to show five, excellent gaits. The assessment of riding ability includes assessment of all gaits under rider with respect to qualities such as correct beat, suppleness, stride length, leg action and speed capacity, as well as the traits general impression and spirit. The ability for collection, self-carriage and lightness in the front part is rewarded. The attributes that are weighed into the scoring of the traits of conformation and riding ability are listed in Table 1, along with the heritability of each trait (Albertsdóttir *et al.*, 2008) and its weight in the total score. The judging scale and the breeding goal within each trait of conformation and riding ability are described in more detail in *Icelandic Horse Breeding* (FEIF, 2014).

Table 1. The traits of conformation and riding ability that are assessed in breeding field tests for Icelandic horses and a list of attributes that are considered in the assessment, the heritability of the traits and weight in the total score.

Trait	Attributes	Weight, %	Heritability
Head	The shape and position of the ears as well as the shape and expression of the head	3	0.29
Neck, withers and shoulders	The length, position and shape of the neck, the height and length of the withers and length and slope of the shoulders	10	0.39
Back and croup	The topline and broadness of the back and the shape, length and inclination in the croup and the muscularity of the back and croup	3	0.29
Proportions	The overall impression and composition of the horse	7.5	0.38
Limbs (quality)	The strength of the limbs and correctness in lateral view	6	0.37
Limbs (correctness)	The correctness of the limbs in frontal and rear view	3	0.22
Hooves	The strength and shape of the hooves	6	0.36
Mane and tail	The thickness and length of the mane and tail	1.5	0.46
Walk	Beat, suppleness, energy and length of strides of the walk	4.0	0.20
Trot	Beat, suspension, movements, length of strides and speed capacity of the trot	7.5	0.38
Gallop	Beat, stretch, suspension and speed of the gallop	4.5	0.36
Canter	Beat, suspension, length of stride, suppleness	-	-
Tölt	Beat, suppleness, length of strides, speed capacity and movements of the *tölt*	15	0.39
Slow *tölt*	Beat, suppleness, length of strides, movements	-	0,38
Pace	Beat, security, movements, suspension and speed of the pace	10	0.58[a]
Spirit	The willingness and disposition of the horse and how sensible and easy it is to handle	9.0	0.37
General impression	The elegance of the horse, head-carriage, raising of the neck and movements of the horse	10	0.31

[a] Refers to estimation using all records.

1.3 Breeding field tests for Icelandic horses

Standardized assessment of Icelandic horses is performed at breeding field tests in more than 10 countries. A panel of three certified breeding judges reaches a consensus on the subjective scoring of the eight conformation traits and seven riding ability traits on a scale of 5.0 to 10.0. Scores are also given for slow *tölt* and canter, which influence the scoring for *tölt* and gallop, respectively, although they are not directly weighed into the total score. A categorical scoring scale is used for the assessment. Additional information is provided by standardized comments on the assessed traits that describe certain attributes of the traits and substantiate the scoring. The minimum age for participation in full assessment is four years, after which the horse can be presented repeatedly. At breeding field tests, the procedure starts with measuring the horses, standing square and evenly on all feet. The measurements include stick measurements: M1 – M5; large calliper measurements: M6 – M8; small calliper measurement: M9; tape measurements: M10 and M11 (Figure 1). All measurements are performed on stallions and six of them on mares (M1, M3, M4, M5, M9 and M10). These measurements are used as an aid for the subjective assessment of the conformation. The conformation is assessed with the horse standing still in the same position as it was when measured, as well as being led in walk and trot back and forth for the assessment of limb correctness. The assessment of the riding ability is the last part of the procedure. It takes place on a straight, level track and is performed in two separate assessments. In the first part of the assessment the horse is presented alone and can be ridden five times in each direction. In the second part, the horse is presented in a group of three horses, it can be ridden three times in each direction and the judges can raise the scores for the traits of riding ability if the horse improves its performance. For participation in the breeding field tests the horse has to be registered and individually identified, healthy and without injuries. The parentage of stallions has to be proven with DNA analysis and their testicles are measured with respect to size and density (FEIF, 2014). In order to decrease the incidence of bone spavin in the population radiographic examination of the distal tarsus is required for stallions before entering the first breeding assessment from the age of 5 years. All information that is collected at breeding field tests is registered in WorldFengur (www.worldfengur.com), the global database for the Icelandic horse.

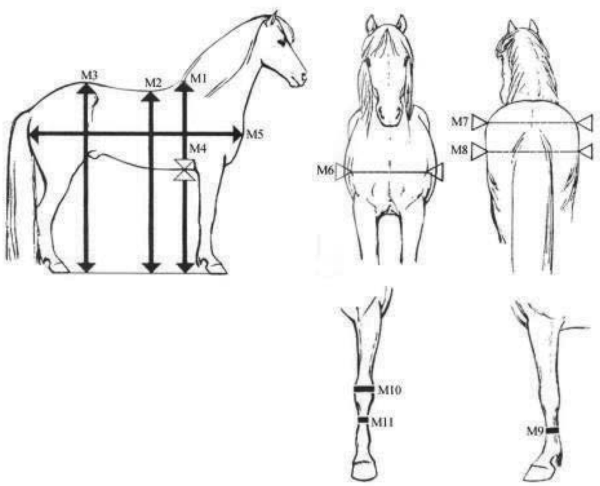

Figure 1. Anatomical landmarks for the standard conformational measurements (M1-M11) (Figures: Pétur Behrens).

1.4 Objective quantification of conformation

The conformation of the horse refers to its form or outline and determines the general appearance of the horse (Saastamoinen & Barrey, 2000). The conformation of horses is traditionally evaluated in a subjective way where individual horses are compared to an ideal. Linear assessment systems have been developed for some horse breeds with the aim of describing where the assessed individual horse lies between the biological extremes for a certain conformational trait (Koenen *et al.*, 1995; Mawdsley *et al.*, 1996). Objective quantification of conformation refers to the assessment of traits such as length of bones, joint angles and deviations of bones from the horizontal or vertical. The aim is to produce reliable and repeatable data that can be related to performance and soundness and used in practice. Direct measurements on the horse (Mawdsley *et al.*, 1996), indirect measurements using photogrammetric methods (Langlois *et al.*, 1978; Holmström *et al.*, 1990; Barrey *et al.*, 2002) and images provided by video records (Crevier-Denoix *et al.*, 2006; Weller *et al.*, 2006a) have been applied. Most of these methods depend on the positioning of the horse in a predefined way and placement of markers on palpable and anatomically interesting landmarks. It has been shown that the placement of markers and stance of the horse are the two main sources of error when measurements are taken live and on photo (Magnusson & Thafvelin, 1990; Weller *et al.*, 2006a). Deviations in camera-horse angle, geometrical errors when a 3-D object is reduced to 2-D image and limited accuracy when measuring conformational parameters manually from photographs (Weller *et al.*, 2006a) can additionally be expected when using photography. Using 3-D images provided by video-records may avoid some of those problems and result in higher accuracy. A 3-D method of morphometric measurements has been developed where the horse is filmed from four angles while walking in a space defined in three dimensions. The method does not require the use of skin markers as the 3-D images allow manual tracking of the anatomical landmarks (Crevier-Denoix *et al.*, 2006). Pourcelot *et al.* (2002) reported that using well defined frames from the walking horse produces more repeatable data than photography as the way of walking is more consistent than the way of standing. Detailed description of the method has been published in a French study and it has been evaluated in terms of repeatability and found to be accurate and to produce repeatable data (Doucet, 2007).

1.5 Association of conformation and performance in horses

The association between conformation and performance has been of interest to horse breeders for centuries. The first text, where conformation was described as an indicator of performance, was written by the Greek Xenophon (430-354 BC). For the past 200 years a substantial amount of literature has been written about conformation of horses and how it relates to performance. The earlier work written for example by Bourgelat (1750) and by Magne (1866) is reported to be of interest as it agrees in many respects with later scientific research, although based entirely on subjective evaluation (Holmström & Back, 2013). In recent years the conformation of horses has been related to performance and soundness using quantitative measurements in a number of studies. They have shown that various conformational aspects are favorable to performance in certain disciplines. Perhaps the most obvious evidence of the effect of conformation on performance is the variability in conformation of breeds that are bred for different purposes. Examples are draught horses, racehorses, dressage and gaited breeds.

Height at withers has been reported to be genetically related to dressage ranking, higher ranking horses being taller (Ducro *et al.*, 2009). Barrey *et al.* (2002) did, however, not find elite dressage horses to be higher than other riding horses. Significant correlations have been found between withers height and kinematic variables, for example a positive correlation between withers height and stride length (Sanchez *et al.*, 2013; Baban *et al.*, 2009; Galisteo *et al.*, 1998). A positive correlation has been shown between height at withers and scores for gaits under rider (Holmström & Philipsson, 1993).

The length of the horse relative to its height at withers has been referred to as the format of the horse. When the length of the body is greater than height at withers the body format is regarded rectangular, compared to a square body format when body length and withers height are about the same. The Arabian horse has been reported to have a square body format, while the general case with the Lippizan horse is a rectangular body format (Zechner *et al.*, 2001). The Swedish Warmblood was reported to have body length three to four cm greater than height at withers (Holmström *et al.*, 1990) and Magnusson & Thafvelin (1990) showed that Standardbred trotters in Sweden had a square body format. Rustin *et al.* (2009) revealed that a rectangular body format had a positive effect on linear traits of walk and trot compared to a square body format; the format of the horse having the highest genetic correlation with stride length in trot. Horses with a short back have been shown to have fewer problems with back pain, but experience more problems with over-reaching in trot

(Magnusson & Thafvelin, 1990). Apparently the effect of body format on performance of gaited breeds has not been studied.

The neck has a major effect on the appearance of the horse. Many of the important attributes of the neck are difficult to quantify objectively, such as the shape and position of the neck, although its length can be objectively measured. Ducro *et al.* (2009) found a relationship between ranking in dressage and neck length in Dutch Warmblood horses; higher ranking horses having longer necks. However, it has been stated that the position of the neck is probably more important than its length in dressage horses (Holmström & Back, 2013).

Lengths, proportions and angles in fore- and hind limbs have been shown to affect performance in a number of ways. Elite horses in dressage and show jumping have been found to have a more sloping shoulder (Holmström *et al.*, 1990) than unselected riding horses. Koenen *et al.* (1995) found a positive genetic correlation of a long sloping shoulder with dressage ability and a sloping shoulder has also been found to correlate with a more protracted forelimb in a study on the correlation of joint angles at square stance to their kinematics at trot (Back *et al.*, 1996). The increased length of the humerus has been found important for larger range of elbow movement. Horses that lift the front hoof higher above the ground and have more protraction in the forelimb and greater carpal flexion at the most forward position of the forelimb, show greater flexion at the elbow joint (Holmström & Back, 2013). A more extended front fetlock joint has been reported to result in more maximal extension which is related to good gait in the forelimb; a suppler strut and more protraction of the forelimb (Back *et al.*, 1994; Back *et al.*, 1996). Long pasterns have been associated with high performing horses and that horses with short upright pasterns are more disposed to injury than horses with long sloping pasterns (Holmström & Back, 2013). A long and sloping front pastern could be better adapted to the storage of elastic strain energy than a short and upright front pastern, resulting in a greater range of movement of the front limb, including more elevated movements.

The importance of the hind limb conformation has been stressed by many authors as the hind limbs provide the horse with propulsive energy and carrying power. A comparison of German and French breeds with the Spanish breed Pura Raza Espaniola revealed the latter to have more closed joint angles in the hind limb (the hip, stifle and hock joint angles). Selection within this Spanish breed has been for elevated and collected gaits (Barrey *et al.*, 2002). Holmström & Philipsson (1993) showed a long and forward sloping femur to be beneficial for scores for gaits under rider in Swedish Warmblood riding horses, as it places the hind limbs further forward under the horse, facilitating function of the hind limbs and improving balance.

In general, there is agreement in the literature that long croup is beneficial for performance but not on its ideal inclination. This could be due to different methods of evaluating croup or pelvis inclination (Holmström & Back, 2013). A sloping croup has been reported to be favorably correlated with movement traits (Koenen *et al.*, 1995), referring to a subjective evaluation of the outer contour of the croup. Based on objective quantification, a flat pelvis was found ideal for dressage ability, as a flat pelvis offers a greater pelvis rotation, resulting in more elastic gaits in dressage horses (Holmström & Back, 2013). A more inclined pelvis and a more flexed hip joint has been shown to place the hind limb more under the horse and decrease maximal retraction of the hind limb, which is essential for collected work in dressage (Back *et al.*, 1996). An adequate slope in the pelvis facilitates the work of the sub-lumbar muscles which tilt the pelvis and bring the hind limbs forward under the horse. Bennett (1992) has pointed out that a more flexed hip joint entails more pelvis rotation, which relates to increased motion in the lumbo-sacral joint and more suppleness at the loins. Holmström *et al.* (1990) found elite dressage and jumping horses to have a larger hock angle than an unselected group of riding horses. They postulated that a hock with a larger angle was more capable of withstanding stress during strenuous work, although it could not be proven that a larger hock angle increased gait quality (Holmström *et al.*, 1990; Holmström & Philipsson, 1993). A small hock joint at square stance has been reported to appear more flexed during stance phase in trot (Back *et al.*, 1996) and characterizes horses that are bred for collected work (Barrey *et al.*, 2002). There is probably no single conformation of the hind limbs that is the ideal one and different combinations of joint angles can have different effects, such as to facilitate maximal pro- and retraction, place the hind limb sufficiently under the horse and thus carry weight, withstand stress and store elastic strain energy (Holmström & Back, 2013).

Reynisdóttir (2001) quantified the conformation of Icelandic horses and found significant relationships of conformational parameters with performance traits. The relationship of 33 conformational parameters with performance traits was assessed. Comparing groups of high class four-gaited and five-gaited horses, a multiple regression of performance traits on these conformational parameters explained over 40% of the variation in performance. These findings suggested that it would be interesting to relate objectively obtained morphometric parameters to riding ability traits in the Icelandic horse. The heritability of measurements is indeed generally higher than the heritability of subjectively scored composite traits (Dolvik & Klemetsdal, 1999; Molina *et al.*, 1999; Suontama *et al.*, 2009) and results encourage the indirect selection for performance through selection for functional conformation (Schröder *et al.*, 2010). This kind of indirect selection is possible if the conformational traits and the performance traits are heritable and genetically correlated in a favorable way (Falconer & Mackay, 1996).

1.6 Effect of the *DMRT3* gene on gaiting ability

In recent years genomic variation has been related to performance of both humans and livestock. In horses sequence variants have been associated with performance in the Thoroughbred horse (Hill *et al.*, 2010a,b; Gu *et al.*, 2010) as well as traits such as size (Makvandi-Nejad *et al.*, 2012), color (Marklund *et al.*, 1996; Brunberg *et al.*, 2006), show-jumping performance (Schröder *et al.*, 2011), temperament (Momozawa *et al.*, 2005) and muscle characteristics and racing aptitude (Petersen *et al.*, 2013). A nonsense mutation in the *DMRT3* gene (*DMRT3_Ser301STOP*) has been reported to have a great impact on gaiting ability in horses (Andersson *et al.*, 2012). The mutation seems to be permissive for the ability to perform lateral gaits, such as *tölt* and pace and homozygosity for the mutation is required but not sufficient for the ability to pace. Moreover, it has a favorable effect on the ability to run fast at trot and pace and seems to inhibit the transition from trot/pace to gallop in a study on Standardbred horses used in harness racing (Anderson *et al.*, 2012). The effect of the mutation on gaiting ability is strong and it has therefore been referred to as the ´Gait keeper´ mutation. Genotyping of different horse breeds demonstrated that the mutant allele (A) is in very high frequency in gaited breeds as well as breeds bred for harness racing, tested non-gaited horse breeds being homozygous for the wild type allele (C). The geographical distribution of the mutation is found worldwide, suggesting it to be an old mutation (Promerová *et al.*, 2014). *DMRT3* is expressed in inhibitory interneurons with projecting ipsi- and contralateral axons that create direct synaptic connections to motor neurons that are present in a special region of the spinal cord (Andersson *et al.,* 2012). A comparison of wild-type and *Dmrt3*-null mice (*Dmrt3* knockout mice) showed that DMRT3 is crucial for the normal development of a coordinated locomotor network and therefore essential for coordinating limb movements (Andersson *et al.,* 2012).

2 Objectives of the thesis

The main objective of the thesis was to assess the effect of conformation and *DMRT3* genotype on riding ability in the Icelandic horse.

The specific aims were:

- Describe and evaluate the use of a three dimensional morphometric method to objectively quantify the conformation of the Icelandic horse and to determine the distribution of conformational parameters and their inter-correlations.
- Evaluate the effect of the *DMRT3* nonsense mutation on the different gaits (quality and speed capacity) in the Icelandic horse and demonstrate how the frequency of the A- and C- alleles has changed in the Icelandic horse population in recent decades.
- Assess the relationship between conformation and riding ability in the Icelandic horse, where both standard conformational measurements and three dimensional morphometric measurements were related to riding ability traits.
- Estimate the heritability of the standard conformational measurements and their genetic correlation with total score for riding ability.

3 Summary of investigations

The thesis summarizes three papers. The first paper includes a 3-D objective quantification of conformation of a group of Icelandic breeding horses. The second paper estimates the effect of the 'Gait keeper' mutation in the *DMRT3* gene on gaiting ability in the Icelandic horse and the development in the frequency of the mutation in the Icelandic horse population for the past decades. The third paper estimates the relationship between standard conformational measurements and riding ability in the Icelandic horse and determines whether 3-D morphometric measurement can discriminate between high-class and low-class horses with respect to scores for the gaits.

3.1 Material

In Paper I 72 breeding horses that were haphazardly selected at breeding field tests in Iceland in the years 2008-2010 were subject to objective quantification in 3-D of conformational traits. Data about the standard conformational measurements, which are routinely performed at the breeding field test, were obtained for these horses from WorldFengur.

In Paper II a sample of 667 Icelandic breeding horses was genotyped. Horses were selected for genotyping on the basis of their scores for the different gaits in breeding field tests. For practical reasons the selection was limited to horses judged in Iceland and Sweden in the years 2000-2012, with a stored DNA sample according to the global database WorldFengur. The data set included 404 five-gaited horses (*pace* score ≥ 5.5) and 263 four-gaited horses (*pace* score=5.0). For estimation of the trend in the frequencies of the *DMRT3* alleles, A and C, in the Icelandic horse population in recent decades, WorldFengur provided a pedigree file containing 410,285 horses (birth-years 1860-2012), of which 706 horses were genotyped.

In paper III records from the standard conformational measurements and assessments of the riding ability were obtained from WorldFengur for all horses presented at breeding field tests in Iceland in the years 2000-2013. The material included in total 20,527 records on 10,091 horses. Calculated conformational traits included: the difference between height at withers and height at back (M1-M2) and the difference between height at withers and height at croup (M1-M3), both referred to as *height at front*; the difference between height at croup and height at back (M3-M2), referred to as *back incline*; the difference between the length of the horse and height at withers (M5-M1) and the difference between length of the horse and height at croup (M5-M3), both referred to as *format of the horse* and the difference between the hip measurements (M7-M8), referred to as *form of the croup*. A subpopulation of 98 horses was haphazardly selected at breeding field tests in Iceland in the years 2008-2010, of which 25 were stallions and 73 mares, and were subject to objective quantification in 3-D of conformational traits. The subpopulation included the 72 horses previously described in Paper I and additionally 26 horses that were included to provide a material with a wider distribution of scores for the different gaits.

3.2 Methods

In Paper I and III a 3-D morphometric method described by Doucet (2007) and Kristjansson *et al.* (2013) was applied. A total of 28 anatomical landmarks were tracked on each horse; for the head and neck, front- and hind part of the horse (see Figures 2-5 for the location of each landmark) and from these, 400 morphometric parameters were automatically calculated. The calculations include variables from direct measurements, such as heights of certain anatomical landmarks, lengths and widths of segments, joint angles, angles of certain body parts with respect to the horizontal and vertical (all these angles are real, calculated in 3-D) and from indirect calculations that are derived from the preceding, such as heights, lengths and widths reported as a proportion of the height at withers or as a proportion of other direct measurements (such as: length of the neck / length of the back) and joint angles projected in the frontal plane (corresponding to the evaluation of body parts from front or behind) and differences such as height at withers minus height at croup, height at base of withers minus height at croup and base of neck relative to height at croup; A8-A10, A9-A10 and T3/A10 (Figure 2).

The tracking of the anatomical landmarks was all performed by the same operator. Three repeatability tests were carried out in order to test the repeatability of the operator. The same side (the same image) was tracked two times for 20 horses for reference images for fore- and hind limbs (Study 1). The left and right sides of 10 horses were tracked two times, again for the reference images for fore- and hind limbs (Study 2). Finally, reference images of the fore- and hind limbs of 10 horses that had been tracked were rotated 180° and then tracked again (Study 3).

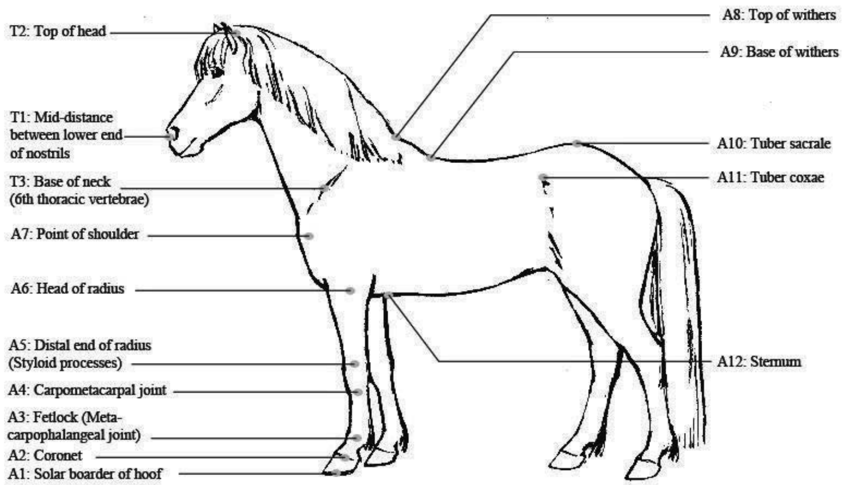

Figure 2. Anatomical landmarks that were tracked for the reference image for the forelimb and the body axis in the 3-D quantification of the conformation. (Figure: Pétur Behrens).

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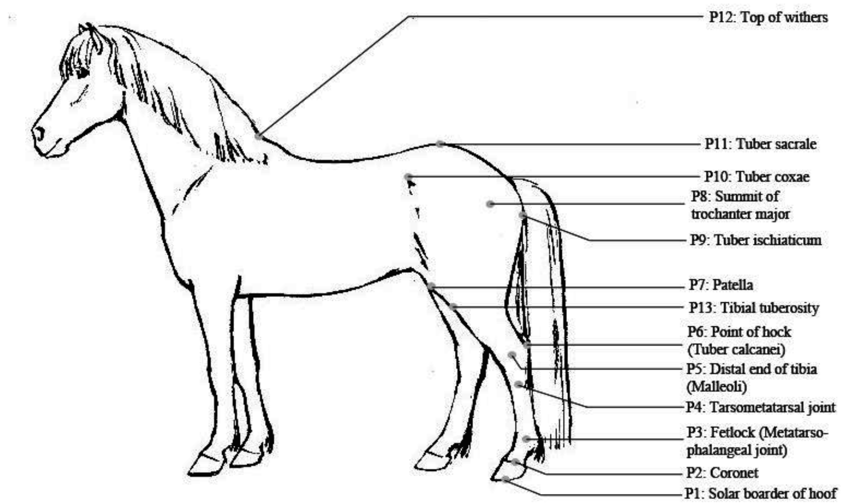

Figure 3. Anatomical landmarks that were tracked for the reference image for the hind limb and the body axis in the 3-D quantification of the conformation. (Figure: Pétur Behrens).

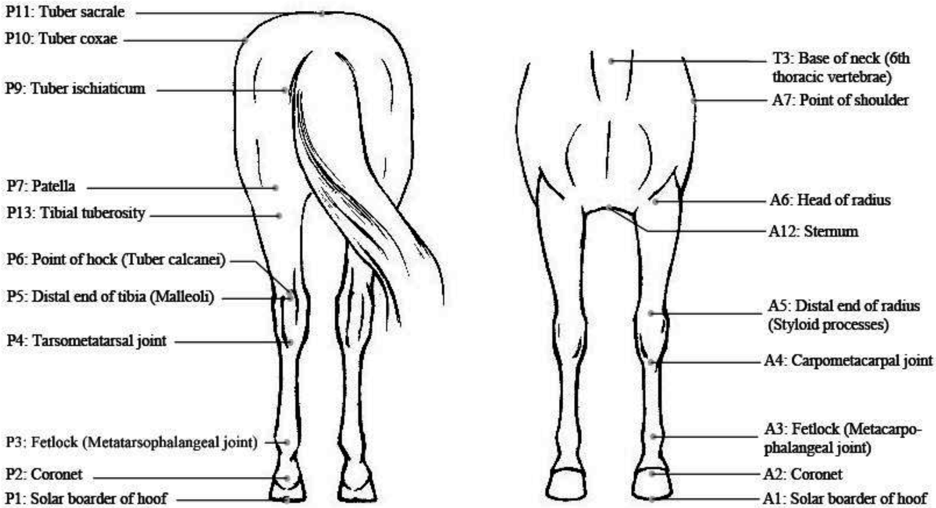

Figure 4. Anatomical landmarks that were tracked for the reference image for the fore- and hind limb and the body axis in the 3-D quantification of the conformation. (Figure: Pétur Behrens).

Figure 5. Anatomical landmarks that were tracked for the reference images for the fore- and hind limb and the body axis in the 3-D quantification of the conformation – depicted on a live horse.

Statistical analysis was mostly carried out using the SAS package (SAS Institute Inc., 2009). In paper I-III the mean, standard deviation (SD), median, skewness and kurtosis were calculated to describe the variation of conformational measurements and riding ability traits within the studied material.

In paper I effects of the age and the sex on the 3-D body measurements were estimated with analysis of variance using PROC GLM (SAS Institute Inc., 2009). The Pearson product-moment correlation coefficient was used to evaluate the relationship between conformation parameters and height at withers. The correlations between length parameters and between length parameters and joint angles/inclines were calculated as partial correlations in order to adjust for the effect of height at withers. The repeatability of the tracking of the 3-D anatomical landmarks was evaluated by calculating the intraclass correlation coefficient, ICC.

In paper II the effects of the age_sex subclasses accounting for the interaction between age and sex and the *DMRT3* genotype of the horse on the gaits were estimated with analysis of variance using PROC GLM (SAS Institute Inc., 2009). A Student´s t-test was used to ascertain whether both scores for *tölt* and age at first evaluation differed significantly between horses with AA and CA genotypes. Then a χ^2 test with 1 degree of freedom (df) was performed in order to study whether proportions of genotypes within a subgroup of 28 horses that had received scores of 9.0-9.5 for *tölt* as four year old deviated significantly from the proportion of the genotypes within the whole data set. Discriminant analysis was performed by using stepwise selection to obtain a subset of the gaits to be able to discriminate between the *DMRT3* genotype classes (AA and C-). Selected gaits were then included in a canonical discriminant analysis to find a linear combination of the gaits that best summarized the difference between the genotype classes. The analyses were performed using the PROC STEPDISC and PROC CANDISC (SAS Institute Inc., 2009), respectively. χ^2 tests with 1 df were performed in order to study whether proportions of genotypes within groups of horses, receiving certain comments from judges describing the gaits, deviated significantly from the proportion of the genotypes within the whole data set.

In Paper II the *DMRT3* genotype probabilities were estimated with a segregation analysis by the Geneprob Fortran program of Kerr & Kinghorn (1996). For this estimation both genotype data and breeding field test scores for pace and trot were used, which provided approximate information on the conditional probabilities of the genotypes. By use of pedigree data and the laws of Mendelian inheritance the preliminary phenotypic scores were updated and improved with the Genotype Elimination (G-E) algorithm of Lange (1997). The accuracy of the genotype probability estimates was evaluated by the genotype probability index (GPI) developed by Kinghorn (1997) to indicate the information content from the segregation analysis. Mean genotype probabilities within each year were the estimate for the genotype frequencies within each cohort and from these the annual development in the frequencies of the A- and C-alleles was plotted for the birth years 1980-2012. A chi-square test with 1 df was performed in order to evaluate whether the genotypes would conform to the Hardy-Weinberg proportions.

In paper III effects of the age_sex subclasses accounting for the interaction between age and sex, year of judgment and a regression effect of each standard conformational measurement (M1-M11) and calculated measurements (see table 7) on the riding ability traits were estimated with analysis of variance using PROC GLM (SAS Institute Inc., 2009). The linearity of the effect of the conformational measurements on the gait traits was tested by including also a quadratic term as a regression parameter in the model.

The DMU package (Jensen & Madsen, 2010) was used to estimate genetic parameters. Variance and covariance components were estimated with bivariate models using average information (AI) algorithm for restricted maximum likelihood, and the asymptotic standard errors of (co)variance components were computed from the inverse of the AI matrix. For the conformational measurements and total score of riding ability the model included the fixed effect of year of judgement, birth-year_sex subclasses, the random additive genetic effect of the horse and the permanent environmental effect of the horse.

The 3-D morpometrical parameters were numerous (400) and intercorrelated. They were therefore subjected to a principal component (PC) analysis to reduce the number of morpometrical parameters to use in further analyses. The first 22 components were retained (eigenvalues ≥1.0) and accounted for 90% of the total variance. The PCs summarised separate groups of common 3-D measurements, namely lengths, widths, angles and proportions within the conformation. The PROC FACTOR (SAS Institute Inc., 2009), which was used for the principal components analysis, also produced the components as standardized scores (linear combinations of the observed 3-D morphometric measurements) for the 22 retained components. These scores were used in further analyses where the 3-D morphometric measurements were related to the gaits. In order to estimate which 3-D morphometric measurements have an effect on the gait traits, the group of 98 horses was divided into low-class and high-class groups containing at least 20 horses with scores of 6.5-7.0 and 9.0-9.5 for the gait traits, respectively. Within pace an additional comparison was made where horses with a score of 5.0 for pace were compared to horses with scores 8.0-9.5 (Mean: 8.45). The horses were also divided into groups containing at least 20 horses on the basis of judges´ comments describing quality and speed capacity of the gaits (FEIF, 2014). The comments that were investigated with regard to 3-D morphometric measurements were: beat, stride length, leg action, suspension, speed capacity and suppleness. The horses used in the comparison groups were all genotyped with regard to the *DMRT3* nonsense mutation (Anderson *et al.*, 2012) and only horses with the AA genotype were used to exclude genotype effect. Discriminant analysis was performed by using stepwise selection to obtain a subset of the 22 PCs that discriminated between the low-class and high class groups within all gaits and the trait general impression. The 3-D morphometric measurements that loaded significantly on the selected PCs were then submitted to canonical discriminant analysis to find a linear combination of the 3-D measurements that best summarizes the difference between the low-class and high-class groups. The analyses were performed using the PROC STEPDISC and PROC CANDISC (SAS Institute Inc., 2009), respectively. Regression models were also developed for the studied riding ability traits using stepwise selection and based on the 22 retained components, for comparison. Information on all the 98 horses was used for this analysis applying PROC REG (SAS Institute Inc., 2009).

4 Main findings

4.1 Objective 3-D quantification of the conformation

In Paper I, the conformation of 72 Icelandic breeding horses is described by 3-D morphometric measurements of heights, lengths and angles, presented in the terms of mean, range and variation. In general the height parameters varied less (CV: 2.17-3.26) than the length (CV: 2.99-10.93) and angle parameters (CV: 1.14-10.03). Age did not significantly affect any of the 3-D morphometric measurements whereas the sex significantly affected eleven measurements; the least square means are listed in Table 2 (adjusted for height at the withers).

Table 2. Least square means of conformation parameters that varied significantly between stallions and mares (adjusted for height at the withers).

Parameters	Stallions	Mares
Height measurements		
Point of shoulder (A7)	92.58	90.95
Elbow (A6)	71.76	69.91
Patella (P07)	82.88	81.45
Point of hock (P06)	52.54	51.44
Length measurements		
Back (A9-A10)	54.06	57.41
Scapula (A7-A8)	55.19	53.63
Width of breast (A7-A7)	34.84	32.66
Depth of breast (A9-A12)	58.15	59.73
3-D joint angles and inclines		
Shoulder joint (A6-A7-A8)	100.71	105.6
Inclination of scapula	53.30	56.64

Deviations were revealed in the correctness of the limbs. Backward deviation of the carpus (calf knees) was commonly seen, as the carpal angle in the lateral view was >180° for 74% of the horses, while 36% had forward deviation of the carpus (bucked knees) with a carpal angle <180° (Mean: 182.89°). In frontal view a medial deviation of the carpus (carpus valgus) was found in all of the horses (except one), demonstrated by a carpal angle <180° (Mean: 174.67°). A fetlock valgus conformation was found in 96% of the horses; front fetlock angle in frontal view <180°. Medial deviation of the tarsus in frontal view, tarsus valgus, was also common as the tarsal angle was <180° in 73% of the horses. Tarsal angle of approx. 180° was found in 19% of the horses while approximately 8% of the horses had a slight lateral deviation of the tarsus, demonstrating tarsus varus. A hind fetlock valgus (hind fetlock angle in frontal view <180°) was found in 93% of the horses, whereas 7% of them were more or less straight (hind fetlock angle in frontal view approx. 180°).

4.1.1 Correlations

The correlation between height at withers and other height parameters were all significant and strong (r>0.5), with the exception of the height of point of hock (r=0.47). The correlation between height at the withers and length parameters were in most cases significant, the correlation being in the range of 0.27-0.42. Height at withers had a significant and positive correlation with the length of the head and back, humerus and depth of the breast, radius, metacarpus, tibia and metatarsus. The height at withers was not correlated with the length of the neck, scapula, croup, femur, fore- and hind pastern or the width of the breast and width of the croup. The height at withers was not correlated with any of the joint angles or inclines. The correlation between lengths, angles and between lengths and angles were also calculated after adjusting for height at the withers. Significant correlations between these conformation parameters are shown in Table 3. Some of the pairs share a marker; for example withers and back share the marker A8. These measurements are therefore not independent and their correlation is influenced by marker placement.

Table 3. Significant correlations of conformation parameters (adjusted for height at the withers).

Parameters	r[a]	CoD[b]
Length measurements		
Withers (A8-A9) - Back (A9-A10)	-0.26	6.76
Scapula (A7-A8) - Back (A9-A10)	-0.44	19.36
Scapula (A7-A8) - Depth of the breast (A9-A12)	-0.26	6.76
Humerus (A6-A7) - Metacarpus (A3-A4)	0.25	6.25
Humerus (A6-A7) - Front pastern (A2-A3)	-0.37	13.69
Metacarpus (A3-A4) - Front pastern (A2-A3)	-0.52	27.04
Femur (P7-P8) - Tibia (P5-P7)	-0.30	9.00
Metacarpus (A3-A4) - Metatarsus (P3-P4)	0.51	26.01
Angle measurements		
Shoulder joint (A6-A7-A8) - Hip joint (P7-P8/P9-P10)	-0.37	13.69
Shoulder joint (A6-A7-A8) - Inclination of femur (P7-P8 w.r.t.h.*)	-0.36	12.96
Shoulder joint (A6-A7-A8) - Front fetlock angle (A2-A3-A4)	-0.24	5.76
Shoulder joint (A6-A7-A8) - Inclination of front pastern (A1-A3 w.r.t.h.*)	-0.27	7.29
Shoulder joint (A6-A7-A8) - Elbow joint (A5-A6-A7)	0.70	49
Inclination of humerus (A6-A7 w.r.t.h.*) - Hip joint (P7-P8/P9-P10)	-0.32	10.24
Inclination of humerus (A6-A7 w.r.t.h.*) - Inclination of femur (P7-P8 w.r.t.h.*)	-0.33	10.89
Elbow joint (A5-A6-A7) - Front fetlock angle (A2-A3-A4)	-0.24	5.76
Inclination of pelvis (P9-P10 w.r.t.h.*) - Hip joint (P7-P8/P9-P10)	0.38	14.44
Inclination of pelvis (P9-P10 w.r.t.h.*) - Tarsus (P3-P4/P5-P7)	-0.25	6.25
Femur to the horizontal plane (P7-P8 w.r.t.h.*) - Inclination of hind pastern (P1-P3 w.r.t.h.*)	0.36	12.96
Hip joint (P7-P8/P9-P10) - Tarsus (P3-P4/P5-P7)	0.39	15.21
Stifle joint (P5-P7-P8) - Tarsus (P3-P4/P5-P7)	0.70	49.00
Stifle joint (P5-P7-P8) - Hind fetlock angle (A2-A3-A4)	0.25	6.25
Length measurements - Angle measurements		
Scapula (A7-A8) - Shoulder joint (A6-A7-A8)	-0.76	57.76
Scapula (A7-A8) - Inclination of scapula (A7-A8 w.r.t.h.*)	-0.68	46.24
Scapula (A7-A8) - Inclination of humerus (A6-A7 w.r.t.h.*)	-0.44	19.36
Humerus (A6-A7) - Inclination of scapula (A7-A8 w.r.t.h.*)	-0.24	5.76
Croup (P9-P10) - Hip joint (P7-P8/P9-P10)	0.26	6.76
Ischium (P8-P9) - Hip joint (P7-P8/P9-P10)	0.68	46.24
Back (A9-A10) - Shoulder joint (A6-A7-A8)	0.56	31.36
Back (A9-A10) - Inclination of scapula (A7-A8 w.r.t.h.*)	0.72	51.84

[a]r = Pearson product-moment correlation coefficient; [b]CoD = coefficient of determination.

4.1.2 Repeatability

The repeatability of the tracking of the anatomical landmarks was tested in three studies. It was concluded that the repeatability was high in most cases. In general the highest repeatability was obtained when the same image was tracked (Study 1). When the left and right sides of the same horse were tracked two times (Study 2) the repeatability indices were in general not as high compared to Study 1. When rotated images of the same horse were tracked (Study 3), an intermediate repeatability was obtained, in general higher than in Study 2. The repeatability of height measurements (anatomical landmarks T3, A3-A10, A12 and P3-P12) ranged from 70.6% to 99.7%. The repeatability for length parameters ranged from 53.3%-96.0%. The least repeatable length parameters in Study 1 were the length of the femur, length of the metatarsus and length of the hind pastern (repeatability: 69.3%, 53.3% and 61.5%, respectively). In Study 2 the length of the femur (71.1%), tibia (53.3%) and metatarsus (71.4%) were the least repeatable anatomical landmarks. In Study 3 the length parameters that were the least repeatable were, as in Study 2, the length of the femur and those associated with the fetlock in front and hind (anatomical landmarks A3 and P3). The repeatability for angle parameters was in the range of 33.9%-98.0%. The angle parameters that had the lowest repeatable in Study 1 were elbow joint (57.0%), hip joint (73.6%) and those associated with the fetlock in front and hind (anatomical landmarks A3 and P3). The angle parameters that had the lowest repeatability in Study 2 (repeatability <50%) were associated with the following anatomical landmarks: Carpometacarpal joint (A4), fetlock (A3) and coronet (A2). Further, the angle parameters that were least repeatable in Study 3 were elbow joint (51.8%) and, as in Study 1, those associated with the fetlock in front and hind (anatomical landmarks A3 and P3).

4.2 Effect of *DMRT3* genotype on gaiting ability

The majority of the 667 horses genotyped for the *DMRT3_Ser301STOP* mutation (N=509, 76.3%), were homozygous for the A-allele (AA) and 150 (22.5%) were heterozygous (CA) while only 8 (1.2%) were found homozygous for the wild type (CC). Among the four-gaited horses, 118 out of 263 (45.0%) were homozygous AA, 137 (52.0%) heterozygous CA and 8 (3.0%) homozygous CC, while 391 out of 404 (96.8%) five-gaited horses were homozygous AA and 13 were heterozygous CA (3.2%). The 13 five-gaited horses with the CA genotype had scores from 5.5-7.0 for pace with a mean score of 5.92, compared to a mean score of 7.30 for horses of the AA genotype. Then, 133 out of the 509 (26.1%) AA horses were presented as four-gaited horses. The *DMRT3* genotype had a significant effect on all gaits except slow *tölt* (Table 4). Scores for walk, trot, gallop and canter were significantly higher among horses with the CA genotype compared to AA horses which had significantly higher scores for *tölt*.

Table 4. Results of analysis of variance for the effect of *DMRT3* genotype on gaits (667 horses). Least square means of homozygous mutant (AA) and heterozygous (CA) horses for scores for gaits. The *P*-values indicate where there is significant difference between least square means.

Trait	**Number of AA**	**Number of CA**	**AA**	**CA**	**p-value**
Walk	502	143	7.52	7.71	*
Trot	509	150	7.99	8.24	***
Gallop	509	150	8.08	8.36	***
Canter	474	119	7.61	8.32	***
Tölt	509	150	8.39	8.26	*
Slow tölt	488	136	8.01	8.04	NS

Levels of significance *=*P*-value<0.05; ***=*P*-value<0.001.

The interaction term between the age_sex classes and genotype (two classes: AA genotype and CA genotype) proved to be non-significant for all gaits except for *tölt*. Stallions aged four and five years with the AA genotype had significantly higher scores for *tölt* than their contemporaries with the CA genotype. Moreover, six year old mares with the AA genotype had significantly higher scores for *tölt* than six year old mares with the CA genotype (Table 5). Mean scores for *tölt* at first evaluation in breeding field tests for CA and AA horses were 8.11 (mean age: 5.5 years) and 8.15 (mean age: 5.1 years), respectively. CA horses were significantly older at first evaluation than AA horses, while the difference in mean score for *tölt* was not significantly different. Further, it was shown that significantly more horses had the AA genotype (93.0%) compared to the CA genotype (7.0%) within a subgroup of 28 horses that had received 9.0 or higher for *tölt* at the age of four years.

Table 5. Results of analysis of variance for the effect of *DMRT3* genotype on *tölt* scores. Differences in least square means of tölt scores between homozygous mutant (AA) heterozygous (CA) horses in different age-sex classes.

Age-sex class	**AA**	**CA**	**Number of AA**	**Number of CA**
Stallion - 4 years	8.55	7.90**	19	10
Stallion - 5 years	8.48	8.18*	72	22
Stallion - 6 years	8.54	8.55	84	27
Stallion - 7 years or older	8.51	8.71	86	37
Mare - 4 years	8.50	8.25	10	2
Mare - 5 years	8.23	8.20	54	10
Mare - 6 years	8.25	7.93*	101	15
Mare - 7 years or older	8.23	8.02	83	27

Levels of significance: **P*-value<0.05; ***P*-value<0.01.

The canonical discriminant analysis revealed the gaits that contributed the most to the divergence between genotype classes. Canter had the highest positive coefficient, followed by gallop and trot; high scores for these gaits indicated a C- genotype. Pace and *tölt* had negative coefficients, with pace having a higher loading value; high scores for these gaits indicated an AA genotype.

The proportions of the AA and CA genotypes differed significantly within groups of horses receiving judges´ comments describing beat in trot, gallop and *tölt*; suspension in trot and gallop and speed capacity and suppleness in *tölt*. Horses with the CA genotype more often had good suspension in trot and gallop, better beat in gallop and were less likely to be four-beated in trot while AA horses were more likely to be supple in *tölt* and to possess good speed capacity in *tölt* (Table 6).

Table 6. The proportion of genotypes within groups of horses receiving certain judges´ comments for the gaits. The *P*-values indicate where the proportions deviate significantly from the expected proportions of 0.76 for the homozygous mutant genotype (AA) and 0.24 for the heterozygous genotype (CA) according to a χ^2 test with 1 degree of freedom.

Trait	Number	AA	C/-	p-value
Walk				
Clear beat	132	0,73	0,27	NS
Trot				
Good speed capacity	123	0,79	0,21	NS
Lack of speed capacity	88	0,83	0,17	NS
Clear beat	131	0,74	0,26	NS
Four-beated	64	0,91	0,09	*
Good suspension	92	0,45	0,56	***
Gallop				
Good speed capacity	166	0,79	0,21	NS
Lack of speed capacity	35	0,83	0,17	NS
Clear beat	65	0,6	0,4	*
Good suspension	77	0,42	0,58	***
Lack of suspension	71	0,96	0,04	***
Tölt				
Good speed capacity	222	0,83	0,17	*
Lack of speed capacity	38	0,53	0,47	***
Clear beat	257	0,78	0,22	NS
Trotty beat	27	0,19	0,81	***
Supple	99	0,87	0,13	*
Stiff	33	0,82	0,18	NS

Levels of significance: **P*-value<0.05; ***=*P*-value<0.001. NS = not significant.

The trend in the frequencies of the *DMRT3* alleles A and C from 1980 to 2012 was estimated in the whole population through calculation of genotype probabilities. The frequency of the A-allele was estimated to be 0.72 in 1980 and 0.94 in 2012 (Figure 6).

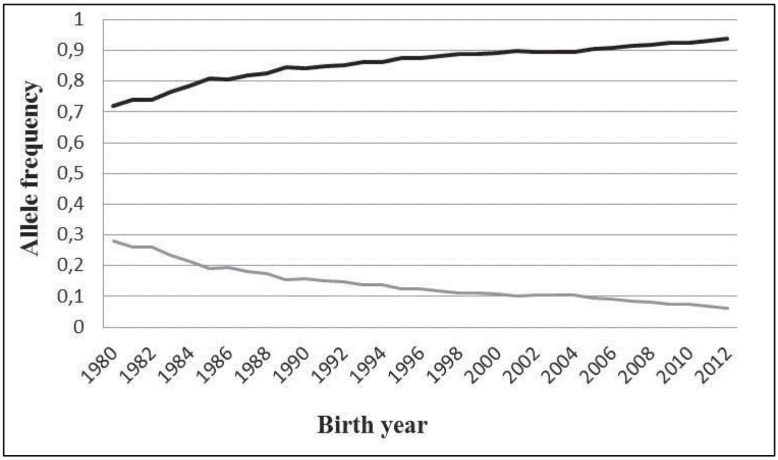

Figure 6. Changes in frequency of the A- and C-alleles in *DMRT3* in the Icelandic horse population from 1980-2012; the black line refers to the A-allele and the grey line refers to the C-allele.

The chi-square test was used to evaluate whether the genotypes reflected Hardy-Weinberg proportions. This was done for each birth year from 1980 to 2012, and the chi-square value regressed on birth year (Figure 7). For the years 1980-1993 the genotypes were not in Hardy-Weinberg equilibrium as the values were above 3.84, which is the 0.05 significance level for one df. In this period the proportion of the CA genotype was higher than expected and the proportion of the homozygotes was subsequently lower. In the years 1994-2012 the genotypes were estimated to be in Hardy-Weinberg equilibrium in the population.

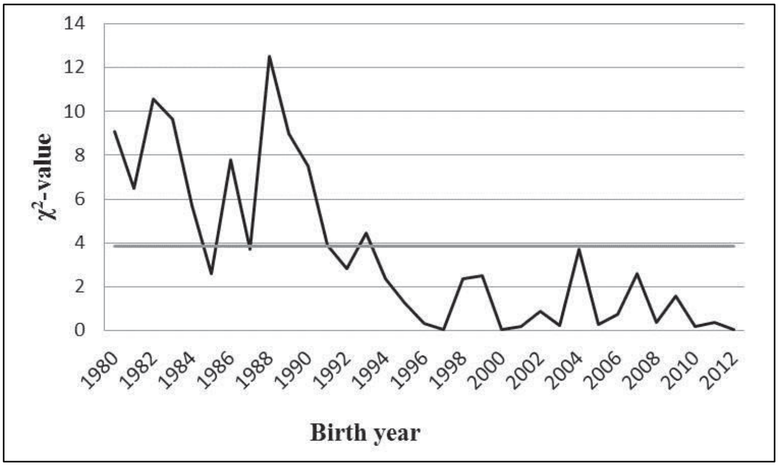

Figure 7. Development of chi-square values over time (black line), testing Hardy-Weinberg equilibrium of *DMRT3* genotypes in the Icelandic horse population, with indicated 0.05 significance level for one degree of freedom (grey line).

4.3 Association of conformation and gaiting ability

The association of conformation and gaiting ability was assessed in two parts. First, the standard conformational measurements and calculated measurements were related to total score for riding ability and each gait. The mean and variation of these measurements can be seen in Table 7, but most of them had a significant phenotypic regression effect on total score for riding ability. Secondly, 3-D morphometric measurements were related to gaiting ability where groups of high-class and low-class horses within each gait trait were compared (Tables 4-11 in Paper III). It was revealed that selected 3-D measurements could discriminate with high accuracy between the groups that were compared.

The main finding for the association of conformation and riding ability was the advantage of an uphill inclination in the horse´s body. The association of the standard and 3-D conformational measurements indicated that a greater difference between front (withers, base of withers, back and base of neck) and hind (croup and *tuber coxae*) was found to be beneficial for gaiting ability. For the standard conformational measurements the optimum difference between height at withers and croup (M1-M3) was 6.2 cm, which was around 2 times higher than the mean value. The optimum difference between height at croup and height at back (M3-M2) was 5.04 cm, which is a fairly low optimum and indicates the disadvantage of a sway back or a back with downhill inclination. The optimum difference between height at withers and height at back of 11.7 cm indicated the advantage of high withers. (Table 7 and

Figures 8-10). The main 3-D morphometric measurements that discriminated between high-class and low-class horses, based on scores for the gaits, were also the ones that describe the height of the horse at front compared to hind: high-class horses had greater height at withers compared to croup, the base of the neck was higher set and higher base of withers compared to croup than in low-class horses.

The phenotypic regression effect of the standard conformational measurements on total score for riding ability was significant in all cases, except height at back and circumference of the carpus and metacarpus (Table 3 in Paper III) and a curvilinear relationship with total score for riding ability was revealed (Figures 8-13). The optimum for the measurements deviated differently from the respective mean values for the material (Tables 1 and 3 in Paper III). For example, the width of the chest and height at withers had a rather high optimum. The phenotypic effect of the measurements on each gait was also studied. The results were similar as for total score, although some discrepancies were found. A difference was revealed in the effect of the format of the horse (M5-M1), where the optimum for *tölt*, trot and pace was -4.3, 3.3 and 2.6 cm, respectively. For the back incline (M3-M2) the quadratic term was not significant for canter and pace where a linear negative relationship was identified. Moreover, the height measurements (M1-M3) did not have a significant effect on scores for pace, nor the measurements M6-M9. The effect of height at withers on the total score among four year old horses was studied specifically (Figure 8).

Several 3-D morphometric measurements discriminated significantly between high-class and low-class horses based on scores for the gaits (Tables 4-11 in Paper III). Height at withers was one of the important measurements separating high-class and low-class in trot, *tölt* and gallop. The length and form of the croup discriminated between the groups in trot, *tölt* and pace. Horses with 8.0 or higher for pace had a more inclined and longer croup (P9->P10/P12) compared to horses with 5.0 for pace; while high-class horses within trot and *tölt* had shorter croups compared to low-class horses. High-class horses in canter and slow *tölt* had a wider pelvis (measured between the hip joint: P8-P8) than low-class horses. Lengths, proportions and joint angles within front and hind limbs discriminated between high-class and low-class horses on the basis of scores for most of the gaits. Length of the front and hind limbs, measured as the height of the head of radius (A6) for the front limbs and the height of the patella (P7) for the hind limbs, discriminated between the groups for walk (relative to height at withers), high-class horses having longer limbs. High-class horses in trot and canter had a longer front pastern relative to the length of the metacarpus than low-class horses, while the opposite was the case for pace. Length of the tibia discriminated between high-class and low-class horses within walk, trot, gallop and *tölt*, high-class horses having longer tibia. The humerus was shorter in high-class horses than low-class horses in walk and trot. High-class horses in walk had a more inclined femur and smaller stifle joint compared to low-class horses. High-class horses in gallop had smaller tarsal angle in lateral view than low-class horses. The inclination of the scapula had an effect on performance in gallop and *tölt* where high-class horses had more inclined scapula. High-class horses in *tölt* had a smaller front fetlock angle in lateral view than low-class horses. The ideal position of the hind limbs as measured as the horizontal distance between the hock and *tuber ischiaticum* varied between canter and pace; a more camped under hind limbs were more ideal for canter, while the opposite was the case for pace. High-class horses within gallop, *tölt* and slow *tölt* had longer necks than low-class horses and high-class horses in trot had a longer back than low-class horses.

The regression models that were developed for each trait of the riding ability based on the 22 retained components gave very similar results as the canonical discriminant analysis and the discrepancies were found unimportant. As depicted by the coefficient of determination (R^2), 3-D morphometric measurements that loaded significantly on the selected components explained 25.1%, 12.2%, 14.4%, 27.5%, 26.7%, 7.7% and 21.8% of the total variance for walk, trot, gallop, canter, *tölt*, slow *tölt* and pace, respectively. Of the 98 horses included in the regressions, 87 were homozygous mutants (AA) with regard to *DMRT3* genotype and 12 were heterozygote (CA). When *DMRT3* genotype was included in the regression models the R^2 increased to 30.0% for pace, for canter it increased to 28.2% and for gallop it increased to 20.3%. The outcome of the regressions for other gaits was not affected by including information on the *DMRT3* genotype.

4.3.1 Heritability of the standard conformational measurements

Estimates of heritability of the standard conformational measurements were moderately high to high (Table 7). The highest estimates were obtained for width of metacarpus and height at withers and the lowest for width of hips (M8) and circumference of metacarpus. The heritability of the calculated measurements was markedly lower than of the standard measurements. The genetic correlation of the standard and calculated conformational measurements with total score for riding ability was in the range of -0.25-0.26. The single measurements had generally lower genetic correlation with the total score for riding ability than the calculated measurements, with the exception of width of the chest and metacarpus.

Table 7. Mean, range and variation of the standard conformational measurements and calculated mearsurements and their heritability (h^2), genetic variance (σ^2_a), permanent environmental variance (σ^2_{pe}), repeatability (t) and genetic correlation with total score for riding ability (r_g).

Parameters:	**Mean**	**SD**	**Range**	h^2	σ^2_a	σ^2_{pe}	**t**	r_g*
Height at withers (M1)	139.64	3.15	126 - 153	0.67	5.92	1.69	0.87	0.06
Height at back (M2)	130.85	2.89	120 - 143	0.58	4.87	2.10	0.83	-0.04
Height at croup (M3)	136.65	2.73	124 - 147	0.65	5.02	1.50	0.85	-0.05
Depth of the breast (M4)	64.17	1.72	54 - 71	0.50	1.49	0.64	0.71	-0.05
Length of the body (M5)	142.50	3.19	128 - 156	0.64	6.89	1.82	0.81	-0.06
Width of the chest (M6)	37.47	1.54	31 - 44	0.40	0.95	0.65	0.67	0.21
Width of the hips (M7)[a]	47.01	1.52	40 - 52	0.54	1.30	0.61	0.79	0.09
Width of the hips (M8)[b]	42.59	1.60	34 - 48	0.37	0.88	0.43	0.55	0.16
Width of metacarpus (M9)	6.55	0.22	6 - 8	0.85	0.14	0.03	1.00	-0.25
Circ. of carpus (M10)	28.20	1.31	23 - 33	0.53	0.37	0.17	0.77	-0.11
Circ. of metacarpus (M11)	17.95	0.81	15 - 22	0.37	0.17	0.10	0.60	-0.03
Height front (M1-M2)	10.25	1.62	2 - 19	0.25	0.65	0.44	0.42	0.25
Height front (M1-M3)	2.99	1.83	-5 - 12	0.27	0.73	0.56	0.47	0.26
Back incline (M3-M2)	6.08	1.52	0 - 17	0.20	0.46	0.58	0.45	-0.08
Format of the horse (M5-M1)	2.86	2.99	-9 - 15	0.40	3.17	1.59	0.60	-0.13
Format of the horse (M5-M3)	5.85	2.78	-6 - 19	0.35	2.67	1.72	0.57	0.00
Form of the croup (M7-M8)	4.42	1.46	0 - 10	0.25	0.50	0.54	0.52	-0.05

*Standard error of genetic correlation between measurements and total score of riding ability ranged from 0.04 to 0.20

[a]Width of the hips between the tuber coxae

[b]Width of the hips between the hip joints

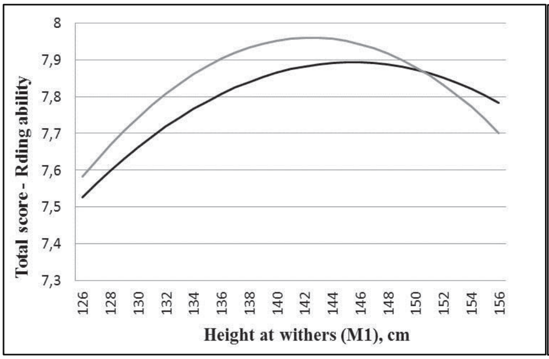

Figure 8. Phenotypic effect of height at withers (M1) on the total score for riding ability. The black line refers to all assessed breeding horses and the grey line refers to four year old horses only.

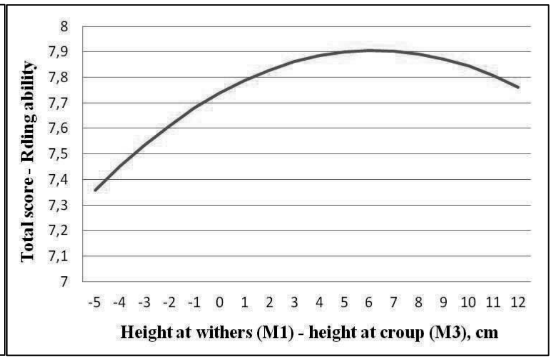

Figure 9. Phenotypic effect of *height at front* (M1-M3) on the total score for riding ability.

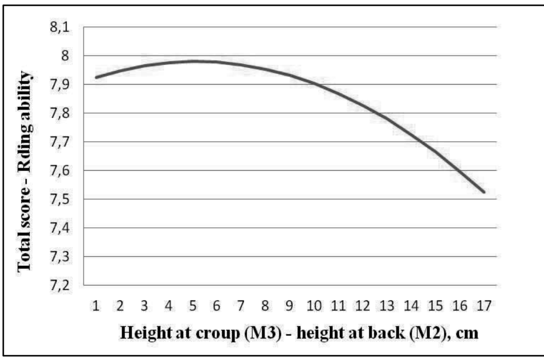

Figure 10. Phenotypic effect of *back incline* (M3-M2) on the total score for riding ability.

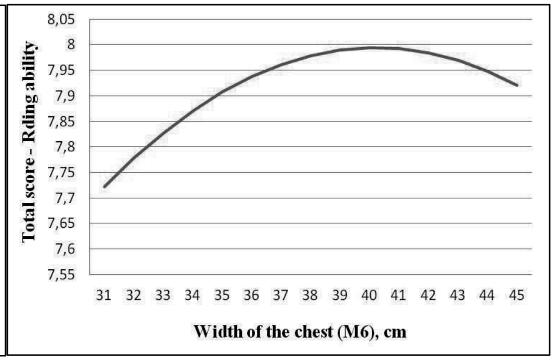

Figure 11. Phenotypic effect of width of the chest (M6) on the total score for riding ability.

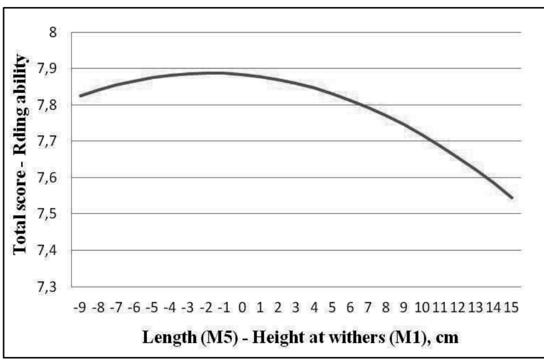

Figure 12. Phenotypic effect of the *format of the horse* (M5-M1) on the total score for riding ability.

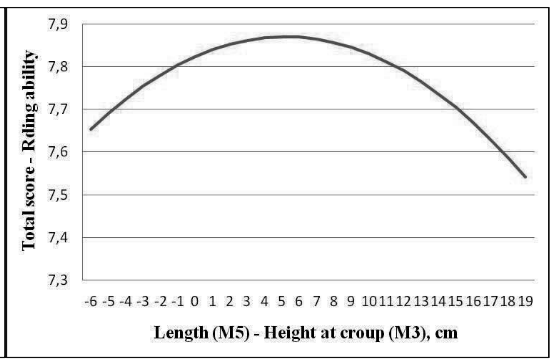

Figure 13. Phenotypic effect of the *format of the horse* (M5-M3) on the total score for riding ability.

4.4 Additional results

Paper III revealed several 3-D morphometric measurements that discriminated significantly between high-class and low-class horses based on scores for all the gaits. The same investigation was also performed for the trait general impression comparing groups of high-class (scores: 9.0-9.5) and low-class (scores: 7.0-7.5) horses. Several 3-D morphometric measurements discriminated significantly between the comparison croups (Table 8), with high confidence as the Mahalanobis distance (D^2) was 5.1 (*P*-value: 0.0162).

The 3-D morphometric measurements could not discriminate between horses based on the studied judges' comments, except for *long strides* in walk, *tölt* and trot. The selected measurements discriminated between horses with rather high confidence as the Mahalanobis distance (D^2) was 1.6 (*P*-value: 0.0341). The horses with the judges´ comment *long strides* were taller (A8), had longer tibia (P5->P78), radius (A3->A6) and humerus (A6-A7), shorter croup (P9-P10), longer front and hind pasterns, and smaller (more extended) front and hind fetlock joint angles (A2->A3, P2->P3 and A2-A3-A4, P2-P3-P4, respectively), compared to horses with no comment regarding stride length or with the comment *short strides*.

The genetic correlation of the standard conformational measurements with the total score for riding ability was presented in Paper III. The genetic and phenotypic correlation between the conformational measurements was also estimated and presented here in Table 9. The standard measurements were also subjected to a principal components analysis, which was performed in same way as for the 3-D measurements (Table 10). The first three components displayed eigenvalues greater than 1 and were therefore retained for rotation. Combined, components one to three accounted for 62% of the total variance. A measurement was said to load on a given component if the factor loading was 0.40 or greater for that component, and was less than 0.40 for the other. Using these criteria, measurements M1 to M5 were found to load on the first component, which was subsequently labeled the size component. Measurements M9 to M11 loaded on the second component, which captured the forelimb bone thickness and measurements M6 to M8 loaded on the third component, which captured widths in the horses´ body.

Table 8. Total canonical structure of the discriminating function separating low-class horses (scores: 6.5-7.0) and high-class horses (scores: 9.0-9.5) within general impression and mean and SD within the comparison groups. (Angles in lateral view).

	Low-class group (N = 20)		High-class group (N = 21)			All horses (n=98)
Parameters	**Mean**	**SD**	**Mean**	**SD**	**Coefficient [a]**	**Mean**
Height at withers (A8)	134,62	2,86	137,15	3,10	0,53	136,23
Length of neck (T2 -> T3)	63,26	3,10	65,64	2,70	0,51	64,87
Tarsus (P3-P4 / P6-P7)	130,84	2,86	128,19	3,78	-0,49	129,18
Height at withers - height at croup (A8-A10)	3,93	2,48	5,65	1,91	0,49	4,74
Length of croup (P9 -> P10)	47,79	1,77	46,69	1,89	-0,39	47,36
Height of head of radius (A6)	70,23	2,20	71,42	2,28	0,35	70,70
Height at croup - height at back (A10-A9)	2,57	2,44	1,42	1,78	-0,35	1,81
Length of tibia (P5 -> P7)	41,74	2,41	42,88	2,26	0,32	42,54
Base of neck / Height at croup (A3/A10)	0,83	0,03	0,84	0,02	0,31	0,82
Shoulder joint (A6-A7-A8)	102,67	5,51	104,92	4,45	0,30	104,90
Inclination of tibia (P7-P6-Horiz.)	44,74	2,76	44,02	3,07	-0,17	44,07
Elbow joint (A5-A6-A7)	142,60	5,09	143,72	3,55	0,17	143,70
Postion of hock w.r.t tuber ischiadicum (horiz. P6 -> P9)	2,60	1,72	2,28	1,53	-0,13	2,45

[a]Absolute canonical coefficients. The mid-point between the group centroid scores, which may be used as a cutting point to assign a previously unclassified horse to a class group, was -0.06; a horse with a value >-0.06 would be classified as a high-class horse and a horse with a value <-0.06 would be classified as a low-class horse.

Table 9. Genetic correlation (above the diagonal) and phenotypic correlation (below the diagonal) between the standard conformational measurements[a].

	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11
Height at withers (M1)	-	0.94	0.94	0.79	0.75	0.46	0.65	0.57	0.49	0.66	0.65
Height at back (M2)	0.54	-	0.96	0.68	0.66	0.40	0.61	0.51	0.28	0.51	0.48
Height at croup (M3)	0.82	0.54	-	0.72	0.78	0.45	0.55	0.54	-	0.68	0.63
Depth of the breast (M4)	0.53	0.25	0.48	-	0.66	0.51	0.64	0.6	0.54	0.60	0.60
Length of the body (M5)	0.55	0.34	0.57	0.48	-	0.53	0.60	0.61	0.53	0.68	0.67
Width of the chest (M6)	0.25	0.27	0.26	0.26	0.29	-	0.61	0.71	0.42	0.51	0.54
Width of the hips (M7)*	0.34	0.20	0.34	0.30	0.34	0.42		0.77	0.37	0.59	0.63
Width of the hips (M8)**	0.10	0.06	0.10	0.07	0.10	0.09	0.11	-	0.54	0.62	0.59
Width of metacarpus (M9)	0.34	0.20	0.32	0.26	0.37	0.23	0.15	-0.15	-	0.74	0.76
Circumference of carpus (M10)	0.46	0.25	0.33	0.21	0.29	0.30	0.30	0.09	0.55	-	0.88
Circumference of metacarpus (M11)	0.42	0.20	0.33	0.29	0.32	0.28	0.29	0.10	0.48	0.76	-

[a]Standard error of genetic correlation between the measurements ranged from 0.00 to 0.25
*Width of the hips between the tuber coxae
**Width of the hips between the hip joints

Table 10. Rotated factor pattern for the standard conformational measurments, M1-M11.

	Components		
	1	2	3
Height at withers (M1)	0.88	0.21	0.11
Height at back (M2)	0.73	0.02	0.06
Height at croup (M3)	0.86	0.21	0.11
Depth of the breast (M4)	0.54	0.27	0.22
Length of the body (M5)	0.64	0.36	0.19
Width of the chest (M6)	0.15	0.36	0.58
Width of the hips (M7)*	0.24	0.28	0.62
Width of the hips (M8)**	0.05	-0.22	0.71
Width of metacarpus (M9)	0.21	0.82	-0.18
Circumference of carpus (M10)	0.29	0.73	0.17
Circumference of metacarpus (M11)	0.15	0.74	0.24

*Width of the hips between the tuber coxae
**Width of the hips between the hip joints

5 General discussion

The main aim of the thesis was to estimate the association of conformation and gaiting ability in the Icelandic horse. The first step was to evaluate a three-dimensional video morphometric method for quantification of conformation. This method was described and applied on a group of Icelandic breeding horses. It was shown that the method produced repeatable data that allowed detailed description of the conformation of the Icelandic horse and was found suitable for further studies. The second step was to estimate the effect of the *DMRT3* 'Gait keeper' mutation on the gaiting ability, using more detailed information and a larger sample of assessed breeding horses than previously published (Andersson *et al.,* 2012). This was done to enable more accurate estimation of the association between conformation and gaiting ability. The third and final step was to assess the effect of conformation on riding ability using both the standard conformational measurements that were giving objective information on a large number of breeding horses, and the 3-D morphometric measurements, enabling a more detailed description of the conformation. The results can be used to improve the subjective scoring of the conformation in the assessment of Icelandic breeding horses at breeding field tests and the use of body measurements. Further, they provide better information to the breeders to apply in practice for facilitating selection decisions.

The descriptive results of Paper I on the 3-D morphometric measurements can probably not be superimposed to the entire population of the Icelandic horse, as assessed breeding horses are preselected with respect to e.g. conformation. It is, however, expected that the estimation of the effect of *DMRT3* genotypes and the conformation on gait quality applies to Icelandic horses in general. The rider effect was not included in the models of the current thesis, although previously shown to have a significant effect on gait quality (Albertsdóttir *et al.,* 2007), because of obvious risk of confounding effects of the rider and genotype /conformation, due to high proportion of riders who rode only one or two horses.

5.1 Objective 3-D quantification of the conformation

The anatomical landmarks of interest were manually tracked and it was therefore essential to test the repeatability of the operator. It was shown that most of the anatomical landmarks could be tracked with high repeatability. The method allowed for fast data acquisition as no skin markers were used on the horses, making it advantageous to use at horse events. The manual placement of anatomical landmarks was, however, too time-consuming for realistic routine application of the method in practice. A more automatic method would therefore be needed for providing the breeders with more detailed information on the conformation compared to the currently available scores for composite traits. Alternatively, a linear scoring scale could be applied, where the conformation is assessed in more detail than the categorical scale used presently for the assessment of Icelandic breeding horses.

The average height at withers of all breeding horses assessed in Iceland in the period 2000-2013 was 139.6 cm. The horses were on average higher at front (height at withers) compared to hind (height at croup) and close to a square body format, as the average difference between length and height at withers were 2.86 cm (Table 7). When these results are compared to the results of Árnason & Bjarnason (1994), who studied the size and body proportions of Icelandic horses in a data set obtained before 1990, it is clear that the Icelandic horse has changed substantially for the past decades. An increase in height at withers from an average of 133 cm (Árnason & Bjarnason, 1994) can partly be explained by longer limbs and higher withers. The proportion of limb length to height at withers has changed from 0.52 in 1994 to 0.55 in 2013 (data not shown). This increase in limb length is probably because of selection of breeding horses with long limbs in this period. Moreover, it is reasonable to believe that improved feeding of Icelandic horses during this period is of importance, as it has been reported that different feeding strategies affect growth (Ringmark *et al.*, 2013). The results demonstrate also that the Icelandic horse has acquired a more uphill conformation, as Árnason & Bjarnason (1994) found the horses to be equally high at withers and croup. When comparing the development in height at croup and height at withers, the increase in height at croup has indeed not been as substantial; the average horse today being around 7 cm higher at withers than reported by Árnason & Bjarnason (1994), compared to 3.6 cm increase in height at croup. This suggests an increase in height of the withers, which is presumably explained by an increase in the height of the spinous processes in the thoracic region and/or a more uphill inclination in the spine. This is regarded as a positive development as it is considered beneficial in the official breeding goal for the Icelandic horse to be higher in front than hind (FEIF, 2014). It is supported by the results of the present study which confirm positive effects on the riding ability. The Icelandic horse has also become shorter and changed from a rectangular body format to a square one. Árnason & Bjarnason (1994) found adult horses to be 10-12 cm longer than their withers height and the average body length to be 145 cm. The present study reveals shorter body format of the Icelandic horse in a data set from 1994 to 2013 (not shown), as the increase in height at withers has been greater than the increase in length. The ideal body format for a high score for *tölt* was a square body format. As *tölt* has the highest weight in the total score, it is suggested that this change in the body format of the

Icelandic horse has been facilitated by the strong selection for *tölt* in the same period. The traits of conformation that have the highest weight in the total score at breeding field tests are the trait neck, withers and shoulder, where high withers are rewarded, and the trait proportions, where long limbs and a light body are rewarded. The greatest genetic gain has been achieved in these two traits representing conformation for the period 1990-2010 (Sigurðardóttir, 2011).

The majority of the horses in this study showed a carpus valgus, as all of the horses (except one) had a carpal angle in frontal view of <180°. In most cases the deviation was within the limits of 10° which is considered to be physiological (Jean-Marie Denoix, personal communication). Then, 74% of the horses had calf knees and 96% had fetlock valgus in front. The results for the hind limbs in frontal view were also quite decisive as most of the horses had tarsus valgus and fetlock valgus. It can therefore be concluded that deviations in the correctness of the limbs are frequent findings in the Icelandic horse. The breeding goal within the trait limb correctness is straight fore- and hind limbs in the frontal view. The smallest genetic gain has been achieved within this trait for the last two decades (Sigurðardóttir, 2011) and it has a rather low weight in the total score (FEIF, 2014). Generally, deviations in limb correctness are considered to have negative influence on health and durability of the horse. For example, lateral deviations in carpal and tarsal joints have been associated with increased risk of tendonitis (the carpal joint) and pelvic fracture and effusion in the digital tendon sheath (the tarsal joint) in National Hunt racehorses (Weller *et al.*, 2006b). Tarsal valgus has been suggested as a risk factor for tarsal osteoarthritis in Icelandic horses (Björnsdóttir, 2002) and the disease has been associated with the tarsal angle in the lateral view (sickle hock conformation) (Axelsson *et al*., 2001). The average horse in this study had a tarsal angle in the lateral view of 140.7° which is smaller than reported by Axelsson *et al.* (2001). This can probably be explained to some degree by different measuring methods and the stance of the horses when measured. The findings confirm that the Icelandic horse has smaller tarsal angles than many other breeds (Holmström *et al.*, 1990; Weller *et al.*, 2006b; Clayton, 2003). The relationship between limb correctness and over-reaching is of concern within the breed. The health aspects of limb correctness needs, however, to be investigated further in the Icelandic horse to evaluate if there is a need to put more emphasis on those traits.

5.2 Effect of *DMRT3* genotype on gaiting ability

A strong effect of *DMRT3* genotype on gaiting ability of Icelandic horses was revealed (Paper II). The *DMRT3* 'Gait keeper' mutation had a favorable effect on the lateral gaits *tölt* and pace. It was confirmed that the AA genotype is a prerequisite for the ability to pace and the results clearly showed a positive effect of the AA genotype on *tölt* ability. This seemed to depend both on superior speed capacity and suppleness of the AA horses compared to the CA horses, attributes that greatly impact the scoring for *tölt* in breeding field tests (FEIF, 2014). The results also indicated that AA horses have more natural ability to *tölt*, as they were significantly overrepresented in a group of breeding horses that received the scores 9.0 or 9.5 at the age of four years. Horses with the CA genotype had on the other hand significantly higher scores for the basic gaits walk, trot, gallop and canter, which confirmed previous results for trot (Andersson *et al.,* 2012). This was related to correct beat and suspension in these gaits, in favor of the CA horses. Based on these results it was concluded the AA genotype reinforces the coordination of ipsilateral limbs, with the subsequent negative effect on the synchronized movement of diagonal limbs. This is supported by previous findings that DMRT3 neurons play a critical role in coordinating limb movement (Andersson *et al.,* 2012). The genotype effect was very strong in canter*,* the C-allele almost being a prerequisite for scores over 9.0 in breeding field-tests (results not shown), which demand correct beat and suspension (FEIF, 2014). Pace has a positive genetic correlation with the other gaits, except canter where the genetic correlation is -0.34 (Elsa Albertsdóttir, personal communication). This reflects the different gaiting ability of AA and C- horses and the presumed effect of *DMRT3* genotypes on the coordination of limb movement. Anderson *et al.* (2012) suggested that the AA genotype promotes speed capacity at trot. This was not supported in the current study, but a possible reason is the tendency of AA horses to become four-beated in trot, allowing higher speed. In Paper II the higher frequency of AA horses among horses with the judges´ comment four-beated trot was revealed. Then it has been suggested that the transition from trot to gallop is triggered when musculoskeletal forces reach a critical level. As gallop is a more compliant gait with the sequential ground contact of limbs, the peak forces are reduced at a certain speed by the transition from trot to gallop (Farley & Taylor, 1991). This critical level could be avoided at high speed in trot by dissociating diagonal limbs (become four-beated) and therefore placing the limbs more sequentially on the ground. It has also been shown that the magnitude of diagonal dissociation at trot increases with speed (Drevemo *et al.,* 1980). Nordic trotters with the AA genotype have been reported to be generally faster in trot than C- horses but not having higher life-time earnings in trot racing compared to CA horses. A possible reason is that the CA horses more often had clear beat in trot and were easier to train, as the AA horses were presumably more often four-beated in trot and moved therefore towards pace (Jäderkvist *et al*., 2014).

The number of CC horses was very low (N=8) in the current data set and those horses appeared to be a diverse group with regard to gait quality, showing for example scores for *tölt* in the range of 6.5-9.0. All tested gaited breed have been reported to carry the 'Gait keeper' mutation in high frequency or in fixation. Possible reasons why CC horses are able to *tölt* have been suggested and include an influential *DMRT3* mutation outside the coding sequence, another gene with great effect on locomotion or a polygenic effect (Promerová *et al.*, 2014). It would have been interesting to relate conformational traits to *tölt* capacity in CC horses. The strong emphasis on *tölt* in breeding of Icelandic horses over many generations could have resulted in conformational traits enabling CC horses to *tölt* to varying degrees and enrichment for mutations that promote *tölt* capacity (Promerová *et al.*, 2014). Then there is a strong tradition in Icelandic equitation to teach all trained horses to *tölt*, although it can be a time-consuming process for C- horses.

The extensive use of the Icelandic horse as a riding horse through the centuries is likely to be the main reason for the preservation of the 'Gait keeper' mutation, but the horses that founded the Icelandic horse population must have carried the mutation. The Icelandic Sagas, in fact, indicate that horses in the early years of the settlement had the ability to pace (Íslendingasagnaútgáfan, 1946). Gaited horse breeds were more common in Europe in earlier times and the use of horses as draught horses used for transport and in agriculture and forestry, combined with a shift in emphasis in breeding and equitation, presumably caused the proliferation of non-gaited breeds (Bennett, 1998). An emphasis in breeding on the basic gaits and a pure beat and suspension in trot and canter could lead to a decrease in the A-allele, as the AA genotype seems to have a negative effect on these attributes.

The estimated increase in the frequency of the A-allele is most likely promoted by the emphasis on lateral gaits, *tölt* and pace, in the breeding goal. Selection of horses with natural ability for *tölt*, reflected by high score for that trait at a young age, can accelerate the marketability of the horse and is therefore celebrated by the breeders. This might also have contributed to the increased frequency of the A allele in the population. The observed trend in the allele frequencies implies that the C-allele may be lost in the Icelandic horse population around year 2030. In light of the favorable effect that the C-allele seems to have on the basic gaits, the breeding goal for the Icelandic horse should perhaps be redefined. Competitions for four-gaited horses are popular and have created a high market value of high-class four-gaited horses (Albertsdóttir *et al.,* 2007). The genotype probability can be estimated with high confidence for many horses. However, the genotyping of four-gaited horses should be of special interest to breeders as they are a mixed group of AA and C- horses and the possible genotypes result in different gaiting ability, which can affect their suitability for the different sport disciplines. In sport competitions for four-gaited horses, attributes such as suspension and clear beat in trot and canter are especially sought-after. Four-gaited stallions that are heterozygous for the 'Gait keeper' mutation are therefore presumably of value in the breeding of excellent four-gaited competition horses. Through genotyping, the mating of two CA horses can be avoided, as there is a 25% chance of a CC offspring with a presumed inaptitude for *tölt*. The breeding of four-gaited AA horses, with quality basic gaits, is likely an obtainable goal and feasible, with the positive effect of the AA genotype on *tölt* in mind. In the years 1980-1993 the *DMRT3* genotypes were not in Hardy-Weinberg equilibrium. The observation of a higher proportion of CA horses than expected from the gene frequencies suggests compensatory mating, where the mating of two four-gaited horses has probably been avoided for this period. The reason for possible reduction in compensatory mating according to *DMRT3* genotypes and a consequent Hardy-Weinberg equilibrium in the population in the years 1994-2012 can probably be explained by decreased selection intensity on the mares' side due to expansion in population size (Sigurðardóttir, 2011), combined with growing popularity of four-gaited horses among which the AA genotype is more common.

5.3 Association of conformation and riding ability

In most breeds of horses the conformation is assessed in order to enhance health, longevity and performance and to breed horses that are aesthetically attractive. In Paper 3 the aim was to estimate the association between the conformation and riding ability of the Icelandic horse. The discriminant analysis was based on genotyped AA horses exclusively to exclude genotype effect. Similar analysis could not be performed for the C-horses as they were too few.

Certain conformational traits were found to have a significant association with riding ability and could discriminate between high-class and low-class horses based on scores for the gaits with high accuracy by multivariate analyses. A study on the genetic correlation between the subjectively scored traits of conformation and gaits in the Icelandic horse has indeed shown correlation in the range of -0.21-0.44 (Albertsdóttir *et al.*, 2008). The results of Paper

III revealed the advantage of high withers and an uphill conformation for the riding ability traits, high-class horses being higher at front than hind compared to low-class horses. It is suggested that uphill conformation promotes collection and self-carriage in the horse and possibly improved balance and increased lightness in the front part. The absolute and relative elevation of the forequarters in relation to the hind part has been regarded as a prerequisite for collection (Heuschmann, 2009), which includes the raising of the base of the neck and the withers, rounding of the back and coiling of the loins. This entails reduction of the weight carried by the forelimbs and increase in the weight carried by the hind limbs (Rodin, 2008; Bennett, 1992). An uphill conformation could facilitate the work of the muscles of the thoracic sling, including the ventral serratus and the pectoral muscles, which lift the rib cage and thereby the withers. Such conformation could also facilitate the work of the scalenus muscles, which lift the base of the neck, and the muscles in the back (longissimus muscles), which assist in the lifting of the forehand (Bennett, 1992; Clayton, 2007¸ Heuschmann, 2009). Good function of these muscles is necessary for self-carriage and collection. High withers have been reported to facilitate the raising of the back (Bennett, 1992). The results of the current study indicate a disadvantage of a forward (downhill) inclination in the back or a sway back. A back incline that is close to the horizontal might be better adapted for force transmission and elevation of the forehand. This was most clearly seen in the relationship between back incline and canter, where a greater degree of collection might be demanded compared to the other gaits. As for the assessment of the composite conformational trait back and croup that is currently used in the breeding assessment (which takes into account the topline in the back), the scoring should probably be done separately for the back and the croup. This would make the assessment more informative as otherwise good attributes of the back can cancel out faults in the croup and *vice versa*. Due to the great impact on riding ability, the weight of the trait back in the total score should be increased as it has a rather low weight today (FEIF, 2014). An effect of training can't, however, be ignored as correct training of for example the sling muscles and back muscles could produce an uphill inclination in the horse´s body to some extent. This needs further investigation in the future.

Height at withers had a significant effect on gaiting ability. The optimum height at withers of 145 cm for the highest total score for riding ability was clearly higher than the average height of 139.64 cm. When the optimum height at croup is compared to mean height at croup, the difference is less, presumably again demonstrating the importance of high withers for riding ability. This effect of the height of the horse on gaiting ability could have two reasons. Firstly, the taller horses usually have longer limbs and therefore longer stride length (Sanchez *et al.*, 2013), and secondly, that height of the horse might have a positive influence on the judges´ impression of the horse, tall horses achieving higher scores for gaits than small horses (Holmström & Back, 2013). When this effect was studied in a group of four year old horses the optimum height at withers was lower, around 142 cm. This could indicate that taller horses need longer training, although age does have a significant effect on height at withers of assessed horses as four year old horses have reached 99.6% of their adult height (Skúlason, 2010).

Rather short body format appeared to be ideal for the riding ability. An optimum difference between length (M5) and height at withers (M1) of -1.7 cm was revealed for the total score for riding ability. This difference varied, however, between the different gaits with the lowest optimum for *tölt*. When the body format was defined as the difference between length of the body and height at croup (M3), the ideal difference was around 5.0 cm. This emphasizes the special importance of high withers for performance in *tölt*. In the subjective scoring of conformation of Icelandic horses a long body format is rewarded and the difference between length of the horse (M5) and height at withers (M1) is used for the assessment (FEIF, 2014). This can have negative impact on the important traits height of the withers and the uphill conformation. To avoid this, the height at the croup should be used in the assessment instead, as it is perhaps a more suitable measure of the size of the horse. The negative effect of increased length of the horse on riding ability could in some cases be explained by a long midsection. Bennett (1992) has for example stated that the lumbar region should ideally be short (and broad), for strength and increased carrying ability of the horse. Then, on the other hand, the midsection should not be too short either. The back must provide enough space for saddle and rider and although a short back has been associated with less back pain, it can lead to increased problems with over-reaching in trot (Magnusson & Thafvelin, 1990). This could explain the advantage of a long body format for trot revealed in the current thesis.

Discrimination analysis based on the 3-D measurements further revealed that high-class horses in walk had longer limbs than low class horses. Stride length is highly rewarded in the assessment of all gaits at breeding field tests for Icelandic horses (FEIF, 2014) and, as previously mentioned; longer limbs can increase stride length. The front pastern was found to be longer in relation to the metacarpus in high-class horses in trot and canter compared to low-class horses, while relatively short pastern was characteristic for high-class horses in pace. This agrees with findings from Swedish Warmblood dressage horses where long pasterns have been associated with elite performance (Holmström & Back, 2013). The front pasterns were found to be more sloping in high-class horses in *tölt*, compared to low-class horses. Long and sloping front pasterns have been related to gait qualities such as stride length and suppleness in other breeds (Back *et al.*, 1994; Back *et al.*, 1996). Horses with short upright pasterns have further been reported to be more disposed to injury (Holmström & Back, 2013), for example, tendonitis of the superficial digital flexor tendon (Weller *et al.*, 2006b). In the assessment of limb quality at breeding field tests for Icelandic horses an intermediate length and slope of the front pasterns is rewarded (FEIF, 2014). This could be re-evaluated in light of these results but limb quality has been reported to have a low genetic correlation with the different gaits (Albertsdóttir *et al.*, 2008). Extremes in ever longer and more sloping pasterns are, however, probably not feasible. At least, short and upright pasterns should probably be avoided, both for performance and durability of the horse. The 3-D morphometric measurements that discriminated between horses with or without the judge´s comment *long strides* indicated the advantage of sloping front pasterns and long limbs, both with regard to length of the radius and tibia and the front and hind pastern. This supported the results that high-class horses in *tölt* and trot had more sloping front pasterns and longer pasterns than low-class horses, respectively, as stride length is highly rewarded in the assessment of these gaits (FEIF, 2014). The reason why 3-D morphometric measurements could not discriminate significantly between horses with other judge´s comments could reflect the inconsistent registration of comments at the breeding field tests and also emphasis the need for objectively obtained kinematic measurements to estimate the relationship between conformation and movements.

The importance of an ideal hind limb conformation has been stated by many authors. For collection the hind-limb should not be camped out, but placed near to the center of gravity, providing more upward than forward propulsion (Clayton, 2013). The current study demonstrated that the hind limbs were more camped under high-class horses than low-class horses in canter as depicted by the position of hock with respect to the *tuber ischiaticum*. This places the hind limbs further under the horse and could therefore create more upward propulsion, which is important for good performance in canter (FEIF, 2014). The pelvis was wider between the hip joints (P8-P8) in high-class horses in canter and slow *tölt*. This was also indicated by the negative linear relationship found between the form of the croup (M7-M8) and total score for riding ability, which suggests that an even croup, and therefore in many instances wide between the hip joints, is beneficial for performance compared to a narrowing croup. This is supported by a broader pelvis having more room for attachment of the gluteal and hamstring muscles that provide propulsion during locomotion (Clayton, 2013) and perhaps placing the hind limbs further apart with the result of improved balance.

There were several 3-D measurements that discriminated between high-class and low-class horses based on scores for the trait general impression. The attributes that are important for high scores for this trait are elegance which is characterized by e.g. good head carriage and raising of the neck, elevated movements and high leg action. The high-class horses were higher at withers, had longer neck and forelimbs. This is not surprising as these conformational traits probably have a positive influence on the elegance of the horse, which is rewarded in general impression. The hind limbs were more camped under the high-class horses, as depicted by less distance between the position of hock and the *tuber ischiaticum*, and smaller tarsal angle in lateral view. This could, as stated, provide more upward than forward propulsion of the hind limbs and therefore more elevated movements. The shoulder and elbow joint angles were larger in high-class horses, with the humerus being more vertical. A large shoulder joint and more vertical position of the humerus has been reported to give higher leg action and increased range of movement for the forelimbs (Bennett, 1992; Holmström & Back, 2013). A more vertical humerus places the forelimb more forward (cranially), which is probably positive for forelimb movements.

The results of Paper II demonstrated that the AA genotype in the *DMRT3* gene is a prerequisite for the ability to pace, although it is clearly not sufficient as 45% of horses classified as four-gaited (pace score=5.0) were homozygous mutants. This group of horses is presumably a mixed group of horses that have no or limited pacing ability. Some of these horses were probably ridden as four-gaited horses although they could perform pace up to a certain level, as it is known that pace training can in some instances impair the *tölt* quality. As *tölt* has the highest weight in the total score and the highest marketing value, good performance in that gait receives highest priority. This high proportion of four-gaited horses with the AA genotype is likely to be the result of genetic, environmental and conformational factors that diminish pace capacity. In Paper III conformational parameters that discriminated between AA horses with 5.0 for pace and good pacing ability were revealed. The importance of an inclined and long croup and a straight back for pacing ability was demonstrated. Moreover, horses with pacing ability had shorter front pasterns and the hind limbs were less camped under than in the four-gaited horses. The advantage of a longer body format for pace was also revealed, compared to the ideal body format for *tölt. Tölt* and pace are very similar gaits, both being in fact four-beat, lateral gaits (Wilson *et al.*, 1998) and only separated by the shorter time between ground contact of lateral limbs and a moment of suspension in pace, which should be non-existing in *tölt* (Zips *et al.*, 2001). The four-gaited AA horses in this study presumably had good speed capacity in *tölt*, demonstrated by a high mean score for *tölt* (results not shown) and the conformational parameters that discriminated between them and the AA horses that had good pacing ability could therefore facilitate suspension at high speed in lateral gaits.

The estimated heritability of the standard conformational measurements was in general high and in accordance with earlier studies (Árnason, 1984a; Dolvik & Klemetdsal, 1999; Suontama *et al.*, 2011). The estimated heritability of the calculated measurements ranged from 0.25-0.40 and was therefore lower than for the direct measurements. The genetic correlation between the standard measurements and total score for riding ability was low, also in accordance with earlier results (Árnason, 1984a). The width of the chest had one of the highest genetic correlation with total score for riding ability (0.21) and it has previously been shown to have significant genetic correlations with kinematic variables (Sanchez *et al.*, 2013). It is suggested that increased width of the chest leads to improved balance, which is a prerequisite for quality gaiting ability (Harris, 1993). This result, combined with the principal components analysis revealing that widths are separate from the size measurements (M1-M5), suggests that the width of the chest should also be measured for the mares and weighted in the assessment of the trait proportions at breeding field tests. Generally, stronger genetic correlations were identified between calculated measurements and total score for riding ability compared to direct measurements, which indicates that the combination of conformational parameters are more important as indicators for performance. Furthermore, estimated heritability of calculated measurements describing the topline of the horse combined with their effect on riding ability designate them as the most important indicators for performance.

The results of the principal components analysis for the 3-D measurements support that variation in the conformation of horses is caused by interactions of many morphometric parameters and cannot be explained by a few underlying components, as pointed out by Weller *et al.* (2006a), considering the high number of retained components. When the standard conformational measurements were subjected to principal components analysis, the first component described size (heights and lengths) and the subsequent two components were related to shape (bone thickness and body widths). This is in accordance with what has been stated by Cadima & Jolliffe (1996) for decomposing morphological variation. Brooks *et al.* (2010) studied morphological variation in a number of horse breeds with a principal components analysis and demonstrated that the first component quantified overall body size and the second component quantified variation in overall bone thickness.

The current thesis has demonstrated that conformation and *DMRT3* genotype can explain a substantial amount of the variation in the riding ability of the Icelandic horse. Although the relationship between the standard measurements and riding ability was assessed with many records, it would have been beneficial to assess the effect of the 3-D measurements in a larger data set containing a larger number of horses heterozygous or homozygous for the *DMRT3* wild type allele. Moreover; it would have been beneficial to be able to relate the 3-D measurements to objectively obtained kinematic variables, instead of the subjective scores for the riding ability traits, to facilitate a more accurate investigation into the effect of conformation on riding ability. In such a research the quality and size of the data set determines how much of the relationship between conformation and performance is revealed. The conformation is decisive for the possible range of movements, as well as high-impact genetic variants such as the 'Gait keeper' mutation which affects the pattern of locomotion. The performance of the horse further depends on the quality of training and the spirit of the horse. The breeding goal for the Icelandic horse is extensive and incorporates five gaits. As indicated by the present results, different conformational attributes are beneficial for performance within the different gaits. The ideal body format for *tölt* was in some aspects different from the ideal one for pace. The ideal conformation of the hind limbs also depends on whether the goal is forward propulsion such as in pace or collection in canter and slow *tölt*. A number of the conformational parameters were although found to be advantageous for all riding ability traits and are believed to benefit the horse regardless of whether it is used for leisure riding or competitions, as qualities such as self-carriage, balance and lightness in the front part are appreciated by all riders.

6 Conclusions

- The 3-D morphometric method provides an objective, repeatable and detailed quantification of conformation and is suited for research purposes but not applicable in practical breeding plan routines, without further technical developments.
- The Icelandic horse has changed substantially during the last decades, with respect to size, body format and proportions.
- Homozygosity for the *DMRT3* ‘Gait keeper’ mutation is permissive for pace and has a major effect on the quality of *tölt*, trot and canter/gallop and speed capacity in *tölt*.
- Selective breeding for lateral gaits in the Icelandic horse population has altered the frequency of *DMRT3* genotypes with a predicted loss of the C-allele in relatively few years.
- A significant curvilinear relationship was revealed between most of the standard conformational measurements and the total score for riding ability.
- Several 3-D conformational measurements could discriminate between high-class and low-class horses based on scores for the different gaits with high accuracy by multivariate analyses.
- An uphill conformation, including height at the withers, was found to be most important for the riding ability. The body format, chest width and lengths, proportions and angles of segments (bones) in fore- and hind limbs had a significant effect on scores for the different gaits.
- These results can be used to improve the assessment of the conformation and consequently the riding ability of the Icelandic horse.

7 Future research

- For better understanding of the relationship between conformation and gait quality, objectively quantified kinematic variables are needed.

- The objective 3-D quantification of the conformation of more horses should be done in order to estimate the heritability of interesting conformational traits.

- The effect of the *DMRT3* genotype and conformation on gait quality should be studied further using performance data from sport competitions.

- The association of proportions in the topline of the horse describing the height at front compared to hind with riding ability calls for further studies, for example on how correct training can influence these proportions.

- An objective quantification of the conformation of young horses should be carried out to study growth and development of Icelandic horses, thus strengthening the selection and culling of untrained horses.

- The association of fore- and hind limb conformation with gait quality should be studied further and related to health issues and durability of the horse. The association of conformation and over-reaching should be studied as well.

- The screening for additional loci that affect gait quality and gaitedness in horses would be interesting, for example, the comparison of CC horses with different ability for *tölt* and AA horses of varying quality in the basic gaits.

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Objective quantification of conformation of the Icelandic horse based on 3-D video morphometric measurements

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ABSTRACT

The official breeding goal for the Icelandic horse describes an ideal conformation that should facilitate multi-gaiting riding ability. The objective of the present study was to describe and evaluate the use of a three dimensional morphometric method to objectively quantify the conformation of the Icelandic horse and to determine the distribution of conformational parameters and their intercorrelations.

Selected material of 72 potential breeding horses attending breeding field tests in Iceland in the years 2008–2010 were recorded while walking in a space which was defined in three dimensions. Four video cameras were used to provide images that allow the determination of 3-D co-ordinates of anatomical landmarks by manual tracking from two or more 2-D views. A set of four video frames was chosen for each horse for two reference images (forelimb/hind limb). The measurements consisted of heights of the anatomical landmarks, segments lengths, joint angles and inclines. Their repeatability was assessed by different repeatability tests.

The study described the conformation of the Icelandic horse in terms of the selected anatomical landmarks. The 3D method provided objective, repeatable data and is suitable for further studies of the correlation between the conformation and other traits as riding qualities and soundness. The method can be applied in other horse breeds. It was confirmed that the Icelandic horse has grown taller in recent years and changed from a rectangular body format to a square one. Measurements of the joint angles of the limbs revealed carpal and tarsal valgus and fetlock valgus to be frequent findings in the breed.

1. Introduction

The conformation of horses has been quantified in a number of studies and horse breeds. The aim has been to describe variation in the conformation (Holmström et al., 1990; Suontama et al., 2009; Weller et al., 2006b) or relate the conformation to performance (Back et al., 1996; Koenen et al., 1995; Langlois et al., 1978; Suontama et al., 2013; Weller et al., 2006c) and soundness (Anderson et al., 2004b; Dolvik and Klemetsdal, 1994). The aim of such studies has also been the development of evaluation methods (Saastamoinen and Barrey, 2000).

Conformation is traditionally evaluated in a subjective way, where the conformation of each horse is compared to an ideal. A linear assessment trait evaluation system is used in some horse populations for conformation evaluation (Koenen et al., 1995; Mawdsley et al., 1996). In recent years methods have been developed that allow objective

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quantification of conformation where the structural arrangement of body segments (the segments lengths and proportions, angles and deviations) are measured with the aim to produce reliable data that can be related to performance and soundness and used in practical breeding work. Both direct measurements on the horse (Mawdsley et al., 1996; Ragnarsson, 1979) and indirect measurements using photography (photogrammetric methods) (Barrey et al., 2002; Holmström et al., 1990; Langlois et al., 1978) and images provided by video records (Crevier-Denoix et al., 2006; Weller et al., 2006a) have been applied. Most of these methods depend on the positioning of the horse in a predefined way and placement of markers on anatomically interesting landmarks. It has been shown that the stance of the horse and placement of markers are the two main sources of error when measurements are taken live and on photo (Magnusson and Thafvelin, 1990; Weller et al., 2006a). Deviations in camera–horse angle, geometrical errors when a 3-D object is reduced to 2-D image and limited accuracy when measuring conformational parameters manually from photographs (Weller et al., 2006a) can additionally be expected when using photography. Using 3-D images provided by video-records may avoid some of those problems and result in higher accuracy. A 3-D method of morphometric measurements has been developed where the horse is filmed from 4 angles while walking. The method does not require the use of skin markers as the 3-D images allow manual tracking of the anatomical landmarks (Crevier-Denoix et al., 2006). It has been reported that using well defined frames from the walking horse produces more repeatable data than photography as the way of walking is more consistent than the way of standing (Pourcelot et al., 2002). Detailed description of the method has been published in a French study and it has been evaluated in terms of repeatability and found to be accurate and to produce repeatable data (Doucet, 2007).

The Icelandic horse is a popular riding horse in Iceland as well as in over 35 other countries. The breeding goal for the Icelandic horse is internationally accepted and Icelandic breeding horses are evaluated by the same breeding standard globally (FEIF, 2012). The official breeding goal for the Icelandic horse describes an ideal conformation for the horse, aimed to facilitate multi-gaiting riding ability and soundness and fulfil certain aesthetic standards. Breeding field tests are arranged for the breeding evaluation where both the riding qualities (7 traits) and the conformation (8 traits) are subjectively judged (FEIF, 2012). Some conformational measurements are also performed and used as an aid for the subjective evaluation. Those have been providing important information about the development of the horse for the last century. The heritability of the traits of conformation and riding ability in Icelandic horses is ranging from 0.22 to 0.46 and from 0.20 to 0.63, respectively (Albertsdóttir et al., 2008; Árnason and Sigurdsson, 2004). Approximately 12.5% of the Icelandic horse population attends the test (Albertsdóttir et al., 2011), representing the future genetic pool.

Limited scientific knowledge concerning the relationship between conformation and performance in Icelandic horses is a drawback for the development of the breed (Andersson et al., 2012). Preliminary studies indicate that reliable quantitative evaluation of conformation might be a prerequisite for such a research. It has been shown in studies dealing with the relationship between conformation and performance in different horse breeds that various conformational aspects are advantageous to performance in certain disciplines (Back et al., 1996; Dolvik and Klemetsdal, 1999; Holmström et al., 1990; Suontama et al., 2013).

The objective of the present study is to describe and evaluate the use of a three dimensional morphometric method to objectively quantify the conformation of the Icelandic horse and to determine the distribution of conformational parameters and their intercorrelations.

2. Materials and methods

2.1. Horses

A total of 72 horses were haphazardly selected at breeding field tests in Iceland in the years 2008–2010 of which 20 were stallions and 52 mares. The age ranged from 4 to 10 years, with a mean of 6.0 years. The total number of sires was 51, with an average of 1.4 offspring per sire. At breeding field tests, the procedure includes measuring the horses, standing square and evenly on all feet. The performed measurements can be seen in Table 1. They include *stick measurements:* M1–M5; *large calliper measurements*: M6–M8; *small calliper measurement*: M9; *tape measurements*: M10 and M11. All measurements are performed on stallions and six of them on mares (M1, M3, M4, M5, M9 and M10). In order to estimate whether these 72 horses would represent the population of breeding horses at the time of filming, means and standard deviations for M1–M11 were calculated for the sample of 72 horses and a reference group of 3164 horses attended breeding field tests in Iceland in the years 2008–2010. A student's *t* test was performed in order to estimate whether there would be a significant difference in the means of these two groups. The results regarding the reference group of horses were further used to study the development of the breed and as a comparison to other breeds (height at withers).

Table 1
Eleven body measurements of 3164 breeding horses shown at breeding field tests in Iceland in the years 2008–2010 (reference group) and a group of 72 breeding horses.

Parameters:	Reference population		Sample of 72 horses	
	Mean	**SD**	**Mean**	**SD**
Height at withers (M1)	140.21	2.94	140.07	2.86
Height at back (M2)	131.37	2.88	130.85	2.50
Height at croup (M3)	137.07	2.63	136.39	2.83
Depth of the breast (M4)	64.32	1.68	64.43	1.51
Length of the body (M5)	142.64	3.07	141.96	3.02
Width of the chest (M6)	37.36	1.56	37.30	1.49
Width of the hips (M7)[a]	47.12	1.69	46.50	1.64
Width of the hips (M8)[b]	42.58	1.58	42.35	0.88
Width of metacarpus (M9)	6.57	0.25	6.56	0.16
Circumference of carpus (M10)	28.23	1.30	28.37	1.29
Circumference of metacarpus (M11)	17.93	0.81	18.08	0.95

[a] Width of the hips between the tuber coxae.
[b] Width of the hips between the hip joints.

2.2. Objective quantification of the conformation using a 3-D morphometric method

2.2.1. Filming

The horses were filmed using Canon MD150 PAL cameras when led by hand at walk on a 1 m wide and 25 m long level track, way and return. The horses were filmed from four views (angles). One camera was at each end of the track, filming the horses from the front and back. Two additional cameras filmed the horses from the side. There was an angle of 60° between the cameras. The height and position of the cameras can be seen in Fig. 1.

Before each session of filming aluminium structure (calibration structure) with 24 junctions (see Fig. 1) was placed in the middle of the field. The structure is 3 m long, 2 m high and 1 m wide. The field of view of the cameras is set to encompass the structure and no more. The structure is then filmed for a few seconds and then removed to allow the passage of horses. This structure has landmarks (24 junctions, not shown on Fig. 1) whose coordinates are known. This is required to calibrate the cameras and facilitates the definition of the space where the horses are in view of the cameras in three dimensions. The horses are then filmed one by one. The goal is to get an image of each horse when it is straight and relaxed and situated in the middle of the recording area. Before each horse the operator performs a gesture which can be seen on all four cameras. This is done in order to be able to synchronise the four films afterwards.

2.2.2. Data processing

A set of three programs was used to process the data, these programs being *Framesaver*, *Calibrater* and *Conformer,* which are described in detail by Pourcelot (2002). Data processing was performed off-line. Each film is digitised with a resolution of 768 by 576 pixels in millions of colours and the four films from the same session are synchronised. This is done using the programme, *Framesaver,* which opens the four films simultaneously and allows the operator to scroll through the films, frame by frame. When the same frame is found on all four films (the synchronising gesture of the operator), the films are stopped and synchronised. After this the four films are run simultaneously. The recording is done with 25 frames per second. There are therefore 4 hundredths of a second between each frame. The synchronisation is thus achieved with an accuracy of plus/minus two hundredths of a second.

Two sets of reference images (RI) are selected and saved for each horse. The RI are selected when the left leg is at the level of the right fetlock while the horse is walking (if the right lateral view is used) for both the fore- and hind limbs. The first sets of video frames that are saved in *Framesaver* are those of the calibration structure. Then frames of horses are saved. A set of four video frames is selected for each horse, corresponding to reference images for the fore- and hind limb (eight frames in total for each horse).

The four video frames of the calibration structure are then loaded with the *Calibrater* programme. In *Calibrater* the space where the horse is in view of the cameras is defined in three dimensions. This is done by superimposing on the images of the calibration structure “skeletons” of the structure. They are formed of squares which represent the 24 junctions of the calibration structure, connected with lines. The squares on the skeleton are placed on corresponding landmarks of the calibration structure after which one obtains four images of the calibration structure with a skeleton superimposed on each of its landmarks. The 2-D co-ordinates on the four images are then known. The coherence of the 2-D co-ordinates of the four images is controlled with the help of a parameter which calculates the mean error. This programme uses a mathematical algorithm called Direct Linear Transformation (DLT). DLT allows for the determination of 3-D co-ordinates of a point from two or more 2-D views of this point (Abdel-Aziz and Karara, 1971). Knowing the 3-D coordinates of the structure and its 2-D coordinates on the image, this method provides a series of mathematical parameters allowing for the calculation of the 3-D coordinates of a point when it has been tracked on at least two images.

Finally, the manual tracking of the anatomical landmarks of interest is performed with the programme *Conformer*. For each horse reference images for the front part are loaded first. Before tracking the anatomical landmarks the images are calibrated with coordinates provided by *Calibrater. Conformer* provides a skeleton with numbered squares corresponding to

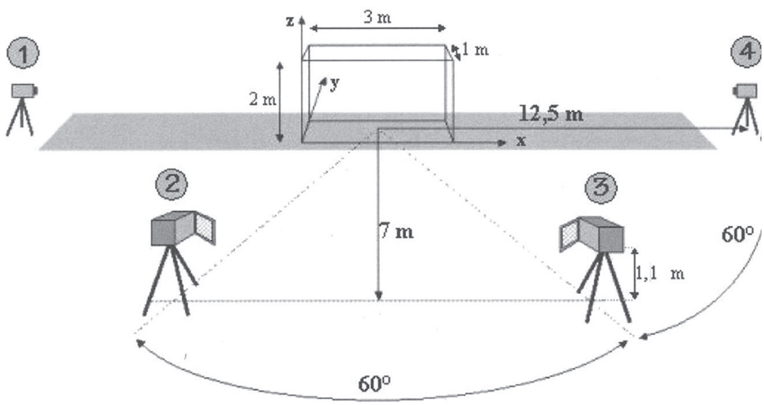

Fig. 1. Position and height of the cameras with respect to the filming area, as well as the size of the calibration structure.

each anatomical landmark of interest. These squares are manually situated on each anatomical landmark. When each square is activated it turns red. As soon as a given landmark is tracked on at least two of the four views, its 3-D coordinates are calculated. They are materialised on the images by a blue square. That allows the operator to monitor the coherence of the tracking at each time. The 3-D coordinates are saved in excel format. The morphometric parameters are automatically calculated from these coordinates and also saved in excel format. The same procedure is performed for the hind part.

2.2.3. Anatomical landmarks

A total of 28 anatomical landmarks were tracked in *Conformer*; for the head and neck, front- and hind part of the horse (see Figs. 2–4 for the location of each landmark). The landmarks were chosen for their anatomical interest as well as their visibility. As no skin markers were used the landmarks have to be visible in order to be able to consistently track them on the images. Therefore it was also necessary to film the horses in bright surroundings (outdoors on sunny days) and have the horses in summer coating. All the tracking of the anatomical landmarks were performed by the same operator.

Conformer automatically calculates 400 morphometric parameters. The calculations are *direct* measurements, such as heights of certain anatomical landmarks, lengths and widths of segments, joint angles, angles of certain body parts with respect to the horizontal and vertical (all these angles are real, calculated in 3-D). Then there are also *indirect* calculations that are derived from the preceding, such as heights, lengths and widths reported as a

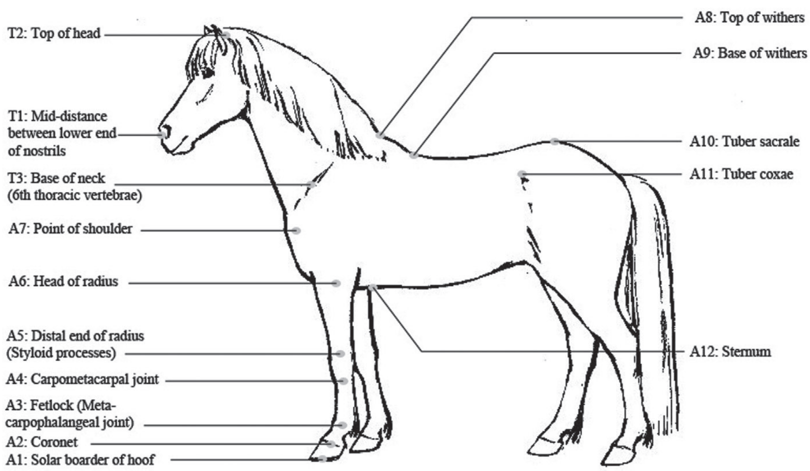

Fig. 2. Anatomical landmarks that were tracked on the reference image for the forelimb.

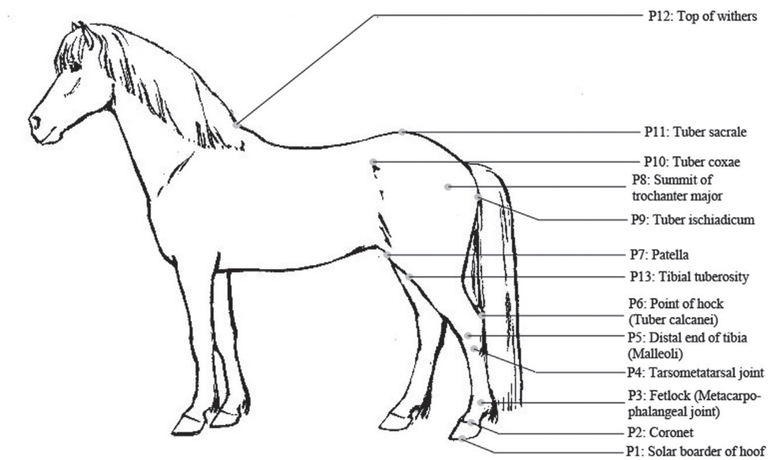

Fig. 3. Anatomical landmarks that were tracked on the reference image for the hind limb.

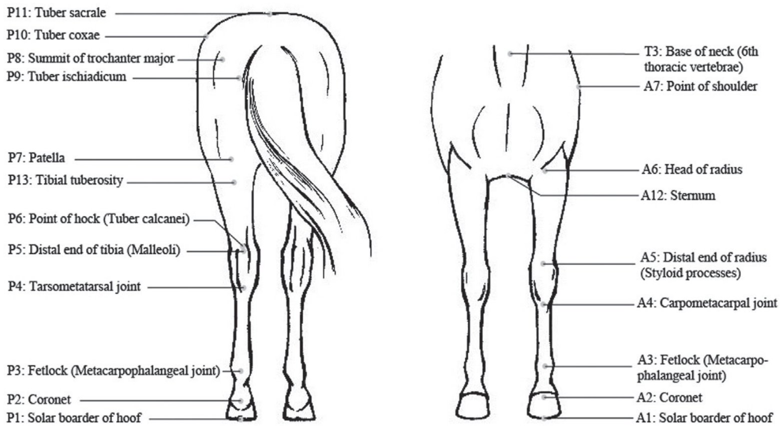

Fig. 4. Anatomical landmarks that were tracked on the reference images for the fore- and hind limb.

proportion of the height at withers or as a proportion of other direct measurements (such as length of the neck/ length of the back) and joint angles projected in the sagittal and frontal planes (corresponding to the evaluation of body parts in profile or from the front or behind).

A carpal angle (lateral view) $>180^\circ$ represents a backward deviation of the carpus (calf knees) and $<180^\circ$ represents a forward deviation of the carpus (bucked knees). As viewed from the front or rear (frontal view), a carpal angle $>180^\circ$ represents a lateral deviation of the carpus (carpus varus) and $<180^\circ$ represents a medial deviation of the carpus (carpus valgus). A fetlock angle $>180^\circ$ represents fetlock varus and $<180^\circ$ represents fetlock valgus. A tarsus angle (frontal view) $>180^\circ$ represents a lateral deviation of the tarsus (tarsus varus) and $<180^\circ$ represents a medial deviation of the tarsus (tarsus valgus).

2.2.4. Repeatability

The tracking of the anatomical landmarks was all performed by the same operator. In order to test the repeatability of the operator, three repeatability tests were performed. The same side (the same image) was tracked two times for 20 horses for reference images for fore- and hind limbs (Study 1). Then the left and right sides of 10 horses were tracked two times, again for the reference images for fore- and hind limbs (Study 2). Here, asymmetry between sides can affect the repeatability and also the fact that the operator is perhaps not working with exactly the same position of the horse. Finally, reference images of the fore- and hind limbs of 10 horses that had been tracked were rotated 180° and then tracked again (Study 3). This was done in order to test whether the direction the horse is facing would affect the tracking; rotated images were used in order to be working with exactly the same image and thereby excluding possible asymmetry between the left and right side of the horse and different stance of the horse.

2.2.5. Data analysis

Statistical analysis was performed using the SAS package (SAS Institute Inc., 2008). The mean, standard deviation (SD), median, skewness and kurtosis were calculated to describe the variation of the 3-D body measurements within the sample of 72 horses. In order to express the relative magnitude of the spread of the parameters the coefficient of variation (CV=SD/mean) was calculated. To test for normality, the Anderson–Darling statistic (Anderson and Darling, 1952) was calculated for the conformation parameters. To ascertain whether the distribution of the 3-D measurements deviates significantly from zero measure of skewness and kurtosis the following calculations were made: Estimated skewness $>1.96\sqrt{6/n}$ for $p<0.05$ and estimated skewness $>2.33\sqrt{6/n}$ for $p<0.01$; and estimated kurtosis $>1.96\sqrt{24/n}$ for $p<0.05$ and estimated kurtosis $>2.33\sqrt{24/n}$ for $p<0.01$.

Effects of the age (four classes: 4, 5, 6 and ≥ 7 years old horses) and the sex (two classes: mares and stallions) on the 3-D body measurements were estimated with analysis of variance using the GLM procedure in the SAS package (SAS Institute Inc., 2008). The following model was assumed for each 3-D body measurement:

$$y_{ijn} = \mu + age_i + sex_j + e_{ijn}$$

where y_{ijn} is a 3-D body measurement for the nth horse, μ is the population mean, age$_i$ is the effect of the ith age group ($i=1,\ldots,4$), sex_j is the effect of the jth sex ($j=1,2$) and e_{ijn} is a random $\sim$NID(0, σ_e^2) residual effect.

The Pearson product–moment correlation coefficient was used to evaluate the relationship between conformation parameters and height at withers. In order to interpret the strength of the relationship the guidelines proposed by Cohen (1988) were used: $r<(-)0.29$=small, $(-)0.30<r>(-)0.49$=medium, $r>(-)0.50$=large. The correlations between length parameters and between length parameters and joint angles/inclines were calculated as partial correlations in order to adjust for the effect of height at withers.

The repeatability of the tracking was evaluated by calculating the intraclass correlation coefficient, ICC, such that ICC$=\sigma_b^2/(\sigma_b^2+\sigma_w^2)$, where σ_b^2 is the inter horse variance and σ_w^2 is the intra horse variance. The proc varcomp procedure in the SAS package (SAS Institute Inc., 2008) was used to produce the inter horse and intra horse variance components.

3. Results

3.1. Mean and variation of the anatomical landmarks

Means and standard deviations of eleven body measurements, both for the reference group of horses and the sample of 72 horses are shown in Table 1. These body measurements are a part of the breeding field test procedure for Icelandic horses. A student's t test revealed no significant difference in the means of these two groups.

The mean, range and variation of the 3-D morphometric measurements for height, length and angle parameters are presented in Table 2. The SD for the height parameters was in the range of 1.68–3.07. The largest SD for the height parameters was observed for the height of the base of neck and the lowest for the point of hock (results not shown). The SD for the length parameters was in the range of 1.13–3.91, the largest SD being for the length of the back. The angle parameters had, with few exceptions, larger SD than the height and length parameters, the range being 2.04–6.42. The largest SD was observed for the shoulder joint in frontal view. In general the height parameters varied less (CV: 2.17–3.26) than the length and angle parameters (CV of length parameters: 2.99–10.93 and of angle parameter: 1.14–10.03).

All the height and length parameters and almost all angle parameters followed a normal distribution, with mean and median being almost identical in all cases. The distribution of the measurements did not deviate significantly from zero

Table 2
Mean and variation of 3-D morphometric parameters in a group of 72 Icelandic horses. Heights and lengths in cm, joint angles and inclines in degrees (angles in lateral view, unless otherwise stated).

Parameters	Mean	SD	Min	Max	CV (%)	Skewness	Kurtosis
Height measurements							
Withers (*A*8)	136.34	2.98	129.89	143.44	2.18	0.17	0.17
Base of withers (*A*9)	129.81	2.83	123.64	136.81	2.18	0.15	−0.39
Croup (*A*10)	131.21	3.09	124.19	139.79	2.35	0.38	0.16
Length measurements							
Head (*T*1–*T*2)	54.92	1.64	51.35	58.60	2.99	−0.01	−0.21
Neck (*T*2–*T*3)	64.94	3.03	56.65	71.68	4.67	−0.20	0.40
Withers (*A*8–*A*9)	20.62	2.24	16.60	26.07	10.85	0.35	−0.53
Back (*A*9–*A*10)	57.44	3.91	47.79	68.00	6.81	−0.05	0.50
Scapula (*A*7–*A*8)	53.97	2.10	48.06	57.72	3.89	−0.29	−0.33
Humerus (*A*6–*A*7)	28.24	1.74	24.07	31.87	6.16	−0.11	−0.26
Radius (*A*5–*A*6)	28.88	1.47	25.29	31.93	5.08	−0.17	−0.61
Metacarpus (*A*3–*A*4)	18.86	1.31	15.86	21.63	6.95	0.15	−0.35
Pastern (*A*2–*A*3)	10.62	1.19	7.18	13.98	11.23	0.05	1.06
Croup (*P*9–*P*10)	47.31	1.96	43.18	53.43	4.18	0.06	0.46
Femur (*P*7–*P*8)	37.68	1.58	32.92	40.47	4.22	−0.51	0.02
Tibia (*P*5–*P*7)	42.27	2.01	38.27	46.98	4.76	0.25	−0.59
Metatarsus (*P*3–*P*4)	25.61	1.25	22.59	28.05	4.89	−0.21	−0.64
Pastern (*P*2–*P*3)	10.32	1.13	7.89	12.98	10.93	0.15	−0.51
Width of breast (*A*7–*A*7)	33.79	3.67	23.24	40.39	10.86	−0.24	−0.22
Width of pelvis (*P*10–*P*10)	43.02	2.96	35.90	49.09	6.89	−0.22	−0.13
Depth of breast (*A*9–*A*12)	59.78	1.97	54.01	64.08	3.30	−0.37	0.09
Depth of breast (*A*8–*A*12)	66.38	2.03	60.08	70.11	3.07	−0.46	0.33
3-D joint angles and inclines							
Shoulder joint (*A*6–*A*7–*A*8)	104.82	5.62	90.60	116.44	5.36	−0.02	−0.38
Shoulder joint (*A*6–*A*7–*A*8)[a]	211.92	6.42	195.43	224.50	3.03	−0.06	−0.44
Inclination of scapula	56.30	3.39	49.06	62.89	6.02	−0.05	−0.57
Inclination of humerus	47.30	3.71	38.68	54.32	7.84	−0.02	−0.48
Elbow joint (*A*5–*A*6–*A*7)	144.24	4.16	132.11	154.65	6.41	−0.15	0.19
Carpus (*A*3–*A*4/*A*5–*A*6)	182.89	5.40	168.93	190.55	2.95	−0.85**	−0.51
Carpus (*A*3–*A*4/*A*5–*A*6)[a]	174.67	2.21	169.13	180.53	1.27	−0.16	0.04
Front fetlock joint (*A*2–*A*3–*A*4)	140.95	5.04	128.20	152.26	3.58	−0.15	−0.41
Front fetlock joint (*A*2–*A*3–*A*4)[a]	173.48	4.50	153.53	181.55	2.59	−1.39**	4.48**
Front pastern inclination	55.07	4.15	41.27	64.31	7.54	−0.32	0.67
Back (*A*8–*A*9–*A*10)	159.5	4.53	148.60	168.82	2.84	−0.39	−0.26
Hip joint (*P*7–*P*8/*P*9–*P*10)	85.43	4.59	74.66	95.38	5.37	−0.31	−0.32
Inclination of pelvis	20.69	2.07	16.04	24.91	10.03	0.05	−0.63
Femur to the horizontal plane	62.99	4.11	53.26	71.99	6.53	−0.19	−0.25
Stifle joint (*P*5–*P*7–*P*8)	119.65	5.66	104.76	133.92	4.73	−0.13	0.53
Tarsus (*P*3–*P*4/*P*5–*P*7)	140.73	3.23	132.21	149.13	2.29	−0.23	−0.03
Tarsus (*P*3–*P*4/*P*5–*P*7)[a]	178.26	2.04	173.42	182.79	1.14	−0.03	−0.64
Hind fetlock joint (*P*2–*P*3–*P*4)	149.91	5.72	139.39	166.07	3.82	0.37	−0.05
Hind fetlock joint (*P*2–*P*3–*P*4)[a]	176.33	2.45	170.25	180.76	1.39	−0.26	−0.32
Hind pastern inclination	55.33	4.58	43.82	65.65	8.29	0.08	0.04

Levels of significance: $^{*}P<0.05$; $^{**}P<0.01$. - Please note * & ** P value should be together and also move the line "Levels of significance: $^{*}P<0.05$; $^{**}P<0.01$" after Angles in frontal view.

[a] Angles in frontal view.

skewness and kurtosis, except carpus (in lateral view) and front fetlock joint (in frontal view) measurements which were not normally distributed.

Age did not significantly affect any of the 3-D morphometric measurements whereas the sex significantly affected eleven measurements; the least square means are listed in Table 3 (adjusted for differing height at the withers).

The average height at withers was 136.34 cm, this being 140.07 cm when the horses were measured with a stick measure as a part of the breeding field test procedure; the average difference being 3.74 cm. The average height at croup was 131.21, compared to 136.39 cm when measured with a stick measure in the breeding field test; the average difference being 5.20 cm. The horses were in all cases higher at withers than at croup, the mean difference being 5.16 cm. The height of the withers, the difference between height at withers (A8) and height at the base of withers (A9), was on average 6.59 cm (range: 3.46–10.15 cm).

In Table 2 the joint angles and inclines of the limbs are listed. Backward deviation of the carpus (calf knees) was commonly seen as the carpal angle in the lateral view was $>180^\circ$ for 74% of the horses, while 36% had forward deviation of the carpus (bucked knees), with a carpal angle $<180^\circ$ (mean: 182.89°). In frontal view a medial deviation of the carpus (carpus valgus) was found in all of the horses (except one) demonstrated by a carpal angle $<180^\circ$ (mean: 174.67°). A fetlock valgus conformation was found in 96% of the horses; front fetlock angle in frontal view $<180^\circ$. Medial deviation of the tarsus in frontal view, tarsus valgus, was also common as the tarsal angle was $<180^\circ$ in 73% of the horses. Tarsal angle of approx. 180° was found in 19% of the horses while approximately 8% of the horses had a slight lateral deviation of the tarsus, demonstrating tarsus varus. A hind fetlock valgus (hind fetlock angle in frontal view $<180^\circ$) was found in 93% of the horses, whereas 7% of them were more or less straight (hind fetlock angle in frontal view approx. 180°).

Proportions between height parameters and height at withers, length parameters and height at withers and between length parameters are presented in Table 4.

Table 3
Least square means of conformation parameters that varied significantly between stallions and mares (adjusted for differing height at the withers).

Parameters	Stallions	Mares
Height measurements		
Point of shoulder (A7)	92.58	90.95
Elbow (A6)	71.76	69.91
Patella (P07)	82.88	81.45
Point of hock (P06)	52.54	51.44
Length measurements		
Back (A9–A10)	54.06	57.41
Scapula (A7–A8)	55.19	53.63
Width of breast (A7–A7)	34.84	32.66
Depth of breast (A9–A12)	58.15	59.73
3-D joint angles and inclines		
Shoulder joint (A6–A7–A8)	100.71	105.6
Inclination of scapula	53.30	56.64

3.2. Correlations

The correlation between height at withers and other height parameters were all significant and strong ($r>0.5$), with the exception of the height of point of hock ($r=0.47$). The correlation between height at the withers and length parameters were in most cases significant, the correlation being in the range of 0.27–0.42. Height at withers had a significant and positive correlation with the length of the head, back, humerus, depth of the breast, radius, metacarpus, tibia, and metatarsus. The height at withers was not correlated with length of the neck, scapula, croup, femur, fore- and hind pastern or the width of the breast and width of the croup. The height at withers was not correlated with any of the joint angles or inclines. Then the correlation between lengths, angles and between lengths and angles was also calculated after adjusting for height at the withers. Significant correlations between these conformation parameters are shown in Table 5.

Some of the pairs share a marker; for example withers and back share the marker A8. These measurements are therefore not independent and their correlation is influenced by marker placement.

Table 4
Proportions between height parameters vs. height at withers, length parameters vs. height at withers and lengths vs. lengths.

Proportions	Mean	SD	Min	Max	CV (%)	Skewness	Kurtosis
Heights vs. height at withers							
Base of neck (T3)/Withers (A8)	0.81	0.02	0.77	0.86	2.21	0.1	−0.2
Back (A9)/Withers (A8)	0.95	0.01	0.93	0.97	1.10	−0.23	−0.24
Croup (A10)/Withers (A8)	0.96	0.02	0.93	1.02	1.80	0.44	0.93
Sternum (A12)/Withers (A08)	0.51	0.01	0.49	0.56	2.27	1.10	2.77
Lengths vs. height at withers							
Neck (T2–T3)/Withers (A8)	0.48	0.02	0.41	0.52	4.87	−0.32	0.08
Croup (P9–P10)/Withers (P12)	0.35	0.02	0.32	0.39	4.70	−0.21	−0.14
Back (A8–A10)/Withers (A8)	0.56	0.03	0.48	0.62	5.08	−0.39	0.24
Width of breast (A7)/Withers (A8)	0.25	0.03	0.17	0.30	10.93	−0.15	−0.34
Lengths vs. lengths							
Head (T1–T2)/Neck (T2–T3)	0.85	0.05	0.74	1.00	5.35	0.79	1.90
Neck (T2–T3)/Back (A8–A10)	0.85	0.06	0.74	1.01	7.65	0.64	−0.04
“Neck” (T2–A8)/Back (A8–A10)	1.00	0.10	0.79	1.20	9.51	0.05	−0.30
Radius (A5–A6)/Metacarpus (A3–A4)	1.54	0.11	1.32	1.81	7.28	0.29	−0.53
Tibia (P5–P7)/Metatarsus (P3–P4)	1.65	0.09	1.44	1.87	5.72	0.03	−0.64

Table 5
Significant correlations of conformation parameters (adjusted for height at the withers).

Parameters	r[a]	CoD[b]
Length measurements		
Withers (A8–A9) – Back (A9–A10)	−0.26	6.76
Scapula (A7–A8) – Back (A9–A10)	−0.44	19.36
Scapula (A7–A8) – Depth of the breast (A9–A12)	−0.26	6.76
Humerus (A6–A7) – Metacarpus (A3–A4)	0.25	6.25
Humerus (A6–A7) – Front pastern (A2–A3)	−0.37	13.69
Metacarpus (A3–A4) – Front pastern (A2–A3)	−0.52	27.04
Femur (P7–P8) – Tibia (P5–P7)	−0.30	9.00
Metacarpus (A3–A4) – Metatarsus (P3–P4)	0.51	26.01
Angle measurements		
Shoulder joint (A6–A7–A8) – Hip joint (P7–P8/P9–P10)	−0.37	13.69
Shoulder joint (A6–A7–A8) – Inclination of femur (P7–P8 w.r.t.h.*)	−0.36	12.96
Shoulder joint (A6–A7–A8) – Front fetlock angle (A2–A3–A4)	−0.24	5.76
Shoulder joint (A6–A7–A8) – Inclination of front pastern (A1–A3 w.r.t.h.*)	−0.27	7.29
Shoulder joint (A6–A7–A8) – Elbow joint (A5–A6–A7)	0.70	49
Inclination of humerus (A6–A7 w.r.t.h.*) – Hip joint (P7–P8/P9–P10)	−0.32	10.24
Inclination of humerus (A6–A7 w.r.t.h.*) – Inclination of femur (P7–P8 w.r.t.h.*)	−0.33	10.89
Elbow joint (A5–A6–A7) – Front fetlock angle (A2–A3–A4)	−0.24	5.76
Inclination of pelvis (P9–P10 w.r.t.h.*) – Hip joint (P7–P8/P9–P10)	0.38	14.44
Inclination of pelvis (P9–P10 w.r.t.h.*) – Tarsus (P3–P4/P5–P7)	−0.25	6.25
Femur to the horizontal plane (P7–P8 w.r.t.h.*) – Inclination of hind pastern (P1–P3 w.r.t.h.*)	0.36	12.96
Hip joint (P7–P8/P9–P10) – Tarsus (P3–P4/P5–P7)	0.39	15.21
Stifle joint (P5–P7–P8) – Tarsus (P3–P4/P5–P7)	0.70	49.00
Stifle joint (P5–P7–P8) – Hind fetlock angle (A2–A3–A4)	0.25	6.25
Length measurements – Angle measurements		
Scapula (A7–A8) – Shoulder joint (A6–A7–A8)	−0.76	57.76
Scapula (A7–A8) – Inclination of scapula (A7–A8 w.r.t.h.*)	−0.68	46.24
Scapula (A7–A8) – Inclination of humerus (A6–A7 w.r.t.h.*)	−0.44	19.36
Humerus (A6–A7) – Inclination of scapula (A7–A8 w.r.t.h.*)	−0.24	5.76
Croup (P9–P10) – Hip joint (P7–P8/P9–P10)	0.26	6.76
Ischium (P8–P9) – Hip joint (P7–P8/P9–P10)	0.68	46.24
Back (A9–A10) – Shoulder joint (A6–A7–A8)	0.56	31.36
Back (A9–A10) – Inclination of scapula (A7–A8 w.r.t.h.*)	0.72	51.84

[a] r = Pearson product–moment correlation coefficient;
[b] CoD = coefficient of determination.

3.3. Repeatability

3.3.1. Study 1

The repeatability when the same image of the same horse was tracked two times (20 horses) was in most cases high to very high. The height of anatomical landmarks T3, A3–A10, A12 and P3–P12 was calculated and the repeatability of these landmarks was very high, ranging from 80.1% to 99.7%. The repeatability for length parameters was in most cases high, ranging from 53.3% to 96.0%, with 50% of the repeatability above 80% and 77% of the repeatability above 70%. The length parameters that were least repeatable were the length of the femur, length of the metatarsus and length of the hind pastern (repeatability: 69.3%, 53.3% and 61.5%, respectively). The repeatability for angle parameters was in the range of 55.8–98.0%, with 50% of the repeatability above 80% and 70% of the repeatability above 70%. The angle parameters that were least repeatable were elbow joint (57.0%), hip joint (73.6%) and those associated with the fetlock in front and hind (anatomical landmarks A3 and P3).

3.3.2. Study 2

When the left and right sides of the same horse were tracked two times (10 horses) the repeatability indices were in general not as high. The height parameters were in all cases highly repeatable, with the repeatability in the range of 70.6–93.8%. The repeatability of the length parameters was in the range of 53.3–95.9%, with 20% of the repeatability above 80%, but 80% of the repeatability above 70%. The length of the femur (71.1%), tibia (53.3%) and metatarsus (71.4%) were the least repeatable anatomical landmarks. The repeatability of the angle parameters was in the range of 33.9–96.4% with 40% of repeatability above 80% and 50% of the repeatability above 70%. The angle parameters that were least repeatable (repeatability $< 50\%$) were associated with anatomical landmarks: Carpometacarpal joint (A4), Fetlock (A3) and coronet (A2).

3.3.3. Study 3

To study better whether the position of the horse has an effect on the tracking, images of 10 horses were tracked and then rotated 180° and tracked again. The repeatability indices were in most cases higher than in Study 2. For the height parameters, the repeatability was in the range of 84.8–99.2%. The repeatability of the length parameters was in the range of 57.6–96.2%, with 50% of the repeatability above 80% and 70% of the repeatability above 70%. The length parameters that were the least repeatable were, as in Study 2, the length of the femur and those associated with the fetlock in front and hind (anatomical landmarks A3 and P3). The repeatability of the angle parameters was in the range of 51.8–92.8%. The angle parameters that were least repeatable were elbow joint (51.8%) and, as in Study 1, those associated with the fetlock in front and hind (anatomical landmarks A3 and P3).

4. Discussion

In this study a three-dimensional morphometric method was described for the first time in English and applied on a group of Icelandic breeding horses. The conformation of the Icelandic horse has not been described in such detail before. The method is operator dependent, as the anatomical landmarks of interest are manually tracked. It was therefore essential to test the repeatability of the operator. It was shown that most of the anatomical landmarks can be tracked with high repeatability and this method is suitable for further studies of the conformation of the Icelandic horse and its relationship with performance and health aspects. The method allows for fast data acquisition as no skin markers are used on the horses, making it advantageous to use at sporting/breeding events. The manual placing of anatomical landmarks is however time-consuming. This method can be applied in other horse breeds but it has been used to quantify the conformation of the Selle Francais horse breed (Crevier-Denoix et al., 2006).

4.1. Mean and variation of the anatomical landmarks

The sample of 72 breeding horses used in the current study is regarded to represent horses that attend breeding shows, as the means of body measurements performed at breeding shows of these horses do not deviate significantly from the means of the reference group of 3164 horses. It was also studied whether the sample of 72 breeding horses would be representative genetically by calculating the genetic relationship of the 72 breeding horses and the reference group of 3164 horses with all horses with the same birth years ($n=62{,}917$). The genetic relationship was exactly the same and it was therefore concluded that the group of 72 breeding horses is also representative genetically (results not shown). Horses that attend breeding shows in Iceland are a preselected group and, hence, the results cannot be superimposed to the entire population. The selection is based on their riding ability and conformation where some traits weigh more into the selection than others. These traits are *neck*, *withers and shoulders*, *proportions*, *toelt*, *spirit* and *form under rider* (Albertsdóttir, 2011).

The 3-D measurements of height at withers and croup are lower than those obtained at the breeding field tests. This is because the horses are standing square and evenly on all feet in the breeding field tests whereas here they are in motion. In terms of the reference image for the forelimbs, the forelimb that is nearer the side view cameras is on the ground with the cannon bone close to vertical, bearing weight, with the other forelimb off the ground. In that moment the pastern sinks and the height at withers therefore gets lower. The same must apply for the hind legs. Comparing values for other similar conformational parameters obtained here and performed at the breeding field tests (comparison of Tables 1 and 2), such as the height at back, depth and width of the breast and width of the pelvis, the values are very similar, the difference being ca. 1–4 cm.

The height parameters varied less than the length and angle parameters. This is in agreement with Holmström et al. (1990) in their study of Swedish Warmblood horses. They found height at withers, back and croup to vary least of the length measurements. Their reported range of coefficients of variation for length and angular measurements is in quite good agreement with the current study, where the inclination of pelvis has the highest coefficient of variation among the angular measurements.

There was no significant effect of age on the 3-D body measurements. This is in agreement with an earlier study which reported that most growth plates in the appendicular skeleton of Icelandic horses were closed by the age of three years (Strand et al., 2007). Árnason and Bjarnason (1994) described the height at withers of Icelandic horses at the age of 4.5 years to be 99.3% of their adult size. The mean age of the horses in the current study was 6.0 years, with 10 horses being 4 years old and the remaining horses 5 years old or older.

Sex of a horse had significant effect on some of the 3-D body measurements. When this effect is compared with the effect of sex in other horse breeds, it must be kept in mind that there are breed differences. Dusek et al. (1981), for instance, reported that the effect of sex on height at withers depends on the breed of the horse. Stallions were higher than mares regarding several height parameters, but Árnason and Bjarnason (1994) and Dolvik and Klemetsdal (1999) reported stallions to have a greater height at withers than mares. Suontama et al. (2009), on the other hand, reported mares to have greater height at withers and croup. The mares had on average longer backs and narrower breasts than the stallions. This corresponds to the findings of Magnusson and Thafvelin (1990) but they reported mares to have longer bodies (and longer distance between the last rib and pelvis) and narrower breasts than the stallions. Suontama et al. (2009) also found mares to have longer bodies than stallions. The mares had significantly deeper breasts than the stallions, but mares have been found to have greater girth circumference than stallions (Dolvik and Klemetsdal, 1999; Saastamoinen, 1990; Suontama et al., 2009). Then the stallions had smaller shoulder joints than the mares and a more inclined scapula.

The conformation of the Icelandic horse has not been quantified before in 3-D. Árnason and Bjarnason (1994) studied growth, development and size of Icelandic horses (measurements made with tape, stick, large and small callipers). Their results were based on data obtained until 1990 of 4882 horses. They reported the size of adult horses, measured with a stick measure, to be about 133 cm, height at croup to be about equal the wither height and the depth of the breast to be 63.9 cm. They found adult horses to be rectangular in shape as they were 10–12 cm longer than their wither height, the average body length being 145 cm. Compared to the reference population of 3164 horses, shown at breeding field tests in the years 2008–2010, the Icelandic horse has become taller in the past 25 years. This increase in height at withers can mostly be explained by longer legs as depth of breast has not changed so much. The average height at withers of the Icelandic horse is 140.1 cm. It is smaller than the Nordic breeds Fjord horses, Finnhorse trotters and Norwegian cold-blooded trotters, but taller than pony breeds such as the Shetland pony and the Welsh pony (Brooks et al., 2010; Dolvik and Klemetsdal, 1999; Suontama et al., 2009). The Icelandic horse has become more quadratic in shape, as the proportion of length vs. height at withers is getting smaller and is 1.02 (the average horse today has height at withers 2.4 cm less than its body length). This is explained by an increase in height at withers and a slight reduction in body length. Icelandic breeding horses are today generally higher in front as the proportion of height at croup to withers height is 0.96. This has changed in recent years compared to the results of Árnason and Bjarnason (1994) where the horses were on average equally high at withers and croup. This is regarded as a positive development as it is considered beneficial in the official breeding goal for the Icelandic horse to be higher in front (FEIF, 2012).

When comparing the results of the present study for segment lengths and angles/inclines to the results of others, it must be kept in mind that the results are dependent upon measuring method and placement of anatomical landmarks. Few comparisons will however be made.

The majority of the horses in this study showed a carpus valgus, as all of the horses (except one) had a carpal angle in frontal view of $< 180^{\circ}$. Then 74% of the horses had calf knees. It can therefore be concluded that carpus valgus and calf knees are characteristic for the Icelandic horse. The same can be said for fetlock valgus conformation in front, as 96% of the horses had fetlock valgus in front. The results for the hind limbs in frontal view were also quite decisive as most of the horses had tarsus valgus and fetlock valgus. For example, in a sample of 108 National Hunt racehorses (Weller et al., 2006b) the majority of the horses had carpus valgus and calf knees, and all of the horses had tarsus valgus. Regarding forelimbs of Norwegian cold-blooded trotters, it has been reported that 25.8% of them were toe-in, 43.9% were toe-out and 36.8% were calf kneed (Dolvik and Klemetsdal, 1999). Then it has been observed that 25% of Finnhorse trotters are toe-out in front (Saastamoinen et al., 1998). It must be kept in mind that different results might be obtained if the conformation of the limbs were studied with the horses standing, as it has been shown that the carpus for example decreases significantly in valgus deformity between standing compared to walking (Unt et al., 2010). It must be noted that the method does probably not in all cases discriminate between a pure deviation and a combination of a deviation and a rotation in the limbs depending on some variation in the localisation of the horse within the chosen frame. The fetlock joint angle (lateral view) was smaller in front than in hind and that is in accordance with the findings of others, the values found here for the Icelandic horse being somewhat lower than found in Standardbred trotters (Magnusson and Thafvelin, 1990) and Swedish Warmblood horses (Holmström et al., 1990). The fetlock joint angle in lateral view can be affected by the height of the heels, high heels making the fetlock angle smaller and low heels making the angle bigger.

The average horse in this sample of horses had a tarsal angle in the lateral view of 140.7°. This value is lower than found for some other breeds, such as the Swedish Warmblood horse (Holmström et al., 1990) and National Hunt racehorses (Weller et al., 2006b). The majority of standing tarsal angles in warmbloods, Andalusians, Thoroughbred, Standardbreds and Arabians was found to fall within the range of 155–165° (Clayton, 2003). Icelandic horses therefore seem to have smaller tarsal angles than many other breeds. This is supported by the findings of Axelsson et al. (2001). They found the mean tarsal angle of 614 Icelandic horses to be around 149° and of all the horses 379 were classified according to Stashak (1987) as being sickle hocked (Axelsson et al., 2001). The difference between the findings of the present study and the findings of Axelsson et al. (2001) is presumable explained by different measuring methods and the stance of the horses; the horses in the study of Axelsson et al. (2001) were standing with limbs placed squarely, the horses in this study are walking, the hind limb being measured taking more weight and the tarsus therefore being more flexed. Axelsson et al. (2001) found horses with larger tarsal angles to have lower prevalence of degenerative joint disease in the distal tarsus. Objective measurements of tarsus valgus have not been reported previously for the Icelandic horse breed.

The conformation of Icelandic breeding horses is evaluated in a subjective way, where each trait is a composite trait. The genetic correlation between the traits of conformation and riding ability (competition traits included) is in the range of -0.24 to 0.54, with most traits having a positive correlation (Albertsdóttir et al., 2008). Reynisdóttir (2001) quantified the conformation of Icelandic horses using the method of Holmström (1990) and assessed the relationship of 33 conformational parameters with performance traits. Comparing chosen groups of high class four-gaited ($n=46$) and five-gaited ($n=43$) horses, a multiple regression of performance traits on these conformational parameters explained over 40% of the variation in performance. These findings suggest that it will be interesting to relate the 3-D morphometric parameters provided by the method applied in the current study to riding ability traits. These parameters (including segment lengths, angles inclines and proportions) give a more thorough and objective description of the conformation than the body measurements and subjective scoring of conformation that is included in the breeding field tests for Icelandic horses today. Then it has been reported that the heritability of measurements is generally higher than the heritability of subjectively scored composite traits (Dolvik and Klemetsdal, 1999; Druml et al., 2008; Molina et al., 1999; Suontama et al., 2009).

4.2. Correlations

The findings of the present study regarding correlations between the conformational parameters are partly consistent with the findings of Weller et al. (2006b) for National Hunt racehorses. They also observed only positive correlations between height at withers and conformation parameters. Further, they found height at withers not to be significantly correlated with angles or inclines and observed a strong correlation with height at withers for croup height. They found a strong correlation with height at withers for back length, scapula length and a moderate correlation for neck and croup length. In the current study, on the other hand, no significant correlation was found between the height at withers and the neck, scapula or croup length and only a moderate, significant correlation for the length of the back. Holmström et al. (1990) reported highly significant regression coefficients in Swedish Warmblood horses for regressions of height at back and croup, width of breast and pelvis and length of neck, scapula, humerus, pelvis and femur on height at withers.

Studying the correlations in Table 5 it is interesting to note the significant correlation between the shoulder joint and hip joint angles, which is in accordance with the significant correlations of inclinations of the humerus and femur. A high negative correlation was observed between length of the scapula and shoulder joint angle; as the scapula is longer, the shoulder joint angle is smaller and the scapula is more sloping. In the subjective scoring of conformation of Icelandic horses a long, sloping scapula is rewarded (FEIF, 2012). A negative correlation was observed between the shoulder joint and the inclination of the pastern. This can probably be explained by possible correlation between the inclination of the humerus and inclination of front pastern, although the correlation was not significant ($r=-0.22$, $P=0.06$). Anderson and McIlwraith (2004a) found a slight, positive correlation between the inclination of the scapula and the inclination of front pastern ($r=0.27$) in 3 year old Thoroughbreds but this was not found here. These findings contradict the common belief that the angle of the scapula and angle of the pastern are equivalent. The inclination of the pelvis was negatively correlated with the tarsal angle, suggesting that as the pelvis gets flatter, the tarsal angle gets larger.

4.3. Repeatability

The repeatability in the present study was high for most of the measurements, especially when the same frame was tracked two times. When comparing repeatability of height parameters to length and angle parameters, there is an indication that the placement of anatomical landmarks in the dorsoventral axis is more repeatable than the placement of landmarks in the craniocaudal axis, as the height parameters have in general a higher repeatability.

The repeatability was in general lower when tracking anatomical landmarks on the same horse using images of the left and right sides. This could be explained by other factors besides operator error. It is possible that the stance (position) of the horse on the reference image for the left and right side was not exactly the same. The reference images were chosen with the horse preferably in the middle of the frame; however, this could have varied between the left and right side images. Possible asymmetry between sides of horses could also have caused error, but asymmetry between sides has been reported in horses by Weller et al. (2006b), Unt et al. (2010) and Manning and Ockenden (1994). The repeatability was especially low (repeatability $<50\%$) for angle parameters in the distal forelimb, when landmarks were tracked on the left and right of the same horse. Angle parameters (in the forelimb) are probably more susceptible to the stance of the horse than height and length parameters (as well as angle parameters in the hind limb). Asymmetry between sides could also be greater in the forelimbs than in the hind limb as Unt et al. (2010) found no significant difference in the hind limb joints between left and right limbs, but found asymmetry between left and right forelimbs and they explained this by the different weight bearing of fore- and hind limbs. Weller et al. (2006a) assessed interoperator and intraoperator repeatability of anatomical marker placement. Interoperator comparison revealed the largest differences in marker placement to be for morphometric parameters associated with the tuber sacrale, the humerus and stifle. Regarding intraoperator repeatability the biggest differences were associated with a marker on the greater trochanter and on the coronet. These findings of Weller et al. (2006a) correspond quite well with the findings of the current project as lower repeatability was observed for the proximal anatomical landmarks: A6 (head of radius) and P8 (summit of trochanter major) and distal anatomical landmarks: A3 and P3 (front and hind fetlock). The lower repeatability for the distal landmarks could be explained by operator error having a greater effect on the measurements as segment lengths are shorter distally, as has been pointed out by Unt et al. (2010).

The repeatability of the method used in the current study has been tested before by Doucet (2007). The same horse was tracked five times over a period of 16 days and the variation of the repeats was calculated. Her findings agree well with the findings of the present study. She found the CV of the repeats was generally well under 5%. Doucet (2007) also evaluated of the accuracy of the method. The average error observed in the determination of the position of a 3-D marker is about 0.55 cm. Under such conditions, the maximum error observed in measuring a distance is 1.10 cm, and the maximum error in measuring an angle (calculated between two segments of 30 cm long) is 2°.

5. Conclusions

The 3-D morphometric method applied in the current study provides an objective and repeatable quantification of conformation and a thorough description of each horse. It allows for fast data acquisition, but the manual placing of anatomical landmarks is time-consuming. It was essential to film the horses in bright surroundings and have them in summer coating in order to consistently track the anatomical landmarks, as no skin markers were used. This method allows for future studies on Icelandic horses where conformation is related to performance and health aspects. The results are quite decisive in their description of joint angles in fore- and hind limbs. The Icelandic horse has changed during the last two decades, both in size and body format.

Conflict of interest

All authors declare that they have no conflicts of interest with this work.

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II

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ORIGINAL ARTICLE

The effect of the 'Gait keeper' mutation in the *DMRT3* gene on gaiting ability in Icelandic horses

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Summary

A nonsense mutation in *DMRT3* ('Gait keeper' mutation) has a predominant effect on gaiting ability in horses, being permissive for the ability to perform lateral gaits and having a favourable effect on speed capacity in *trot*. The *DMRT3* mutant allele (A) has been found in high frequency in gaited breeds and breeds bred for harness racing, while other horse breeds were homozygous for the wild-type allele (C). The aim of this study was to evaluate further the effect of the *DMRT3* nonsense mutation on the gait quality and speed capacity in the multigaited Icelandic horse and demonstrate how the frequencies of the A- and C- alleles have changed in the Icelandic horse population in recent decades. It was confirmed that homozygosity for the *DMRT3* nonsense mutation relates to the ability to pace. It further had a favourable effect on scores in breeding field tests for the lateral gait *tölt*, demonstrated by better beat quality, speed capacity and suppleness. Horses with the CA genotype had on the other hand significantly higher scores for *walk, trot, canter* and *gallop,* and they performed better beat and suspension in *trot* and *gallop*. These results indicate that the AA genotype reinforces the coordination of ipsilateral legs, with the subsequent negative effect on the synchronized movement of diagonal legs compared with the CA genotype. The frequency of the A-allele has increased in recent decades with a corresponding decrease in the frequency of the C-allele. The estimated frequency of the A-allele in the Icelandic horse population in 2012 was 0.94. Selective breeding for lateral gaits in the Icelandic horse population has apparently altered the frequency of *DMRT3* genotypes with a predicted loss of the C-allele in relatively few years. The results have practical implications for breeding and training of Icelandic horses and other gaited horse breeds.

Introduction

One of the major characteristics of horse breeds is their ability to perform specific gaits. A gait is a coordination pattern of the limbs identified by timing and sequence of the footfalls. The gait chosen by a horse depends on speed, genotype and environmental factors (Alexander 1988; Clayton 2004). The Icelandic horse is a multigaited horse breed showing the standard gaits of all domestic horse breeds that are *walk*,

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trot, *canter* and *gallop*. In addition, it has *tölt* and *pace*. *Tölt* is a four-beat running gait with lateral sequence of footfalls and without suspension. *Pace* is considered a two-beat gait with a moment of suspension where lateral legs move almost synchronously back and forth and is optimally a very fast gait. Icelandic horses that possess *walk, trot, canter, gallop* and *tölt* are referred to as four-gaited horses, whereas horses that additionally have the ability to perform *pace* are called five-gaited horses.

A nonsense mutation in *DMRT3* (*DMRT3_Ser301-STOP),* also referred to as the 'Gait keeper' mutation, has been shown to have a great impact on gaiting ability in horses (Andersson *et al.* 2012). Previous work has indicated that the mutation is permissive for the ability to perform lateral gaits, such as *tölt* and *pace*, and homozygosity for the mutation is required although not sufficient for the ability to pace. Moreover, the mutation was reported to have a favourable effect on speed capacity in *trot* and seemed to inhibit the transition from *trot* to *gallop* in a study on Standardbred horses used in harness racing (Andersson *et al.* 2012). The *DMRT3* mutant allele (A) was found in high frequencies in gaited breeds and breeds bred for harness racing, while tested non-gaited horse breeds were found homozygous for the wild-type allele (C) (Andersson *et al.* 2012). Comparison of wild-type and *Dmrt3*-null mice showed that DMRT3 is crucial for the normal development of a coordinated locomotor network that controls limb movement. It was concluded that DMRT3 neurons are essential for left/right coordination as well as for coordinating the movement of fore- and hind legs (Andersson *et al.* 2012).

The Icelandic horse is bred for leisure riding as well as for sport competitions (Albertsdóttir *et al.* 2007; FIZO 2012), with the international breeding goal for the Icelandic horses promoting five-gaited horses. The breeding assessment system is based on breeding field tests for both riding qualities and conformation, where assessment of riding qualities includes judging of the five gaits (FIZO 2012). Scores are also given for *slow tölt* and *canter* although they are not weighed into the total score, but influence the scoring for *tölt* and *gallop*, respectively. The horses are judged on a scale from 5 (not presented) to 10 (best) with intervals of 0.5, the average being 7.5. The judges can also give standardized comments on the assessed traits that describe certain attributes of the traits and substantiate the scoring (listed in Table S1 for the five gaits). Horses can only receive scores above average if they present one or more of the listed advantages and horses below average have one or more of the listed disadvantages. Horses can attend the breeding field tests from the age of four, the majority being five and 6 years old. Approximately 12.5% of the Icelandic horse population is presented based on a preselection by the breeders (Albertsdóttir *et al.* 2011).

The aim of this study was to evaluate the effect of the *DMRT3* nonsense mutation on the gait quality and speed capacity in the multigaited Icelandic horse and demonstrate how the frequency of the A- and C-alleles has changed in the Icelandic horse population in recent decades.

Material and methods

Estimation of DMRT3 *genotype effect on gait traits*

Selection of horses

Horses were selected for genotyping on the basis of their scores for the different gaits in breeding field tests. For practical reasons, the selection was limited to horses judged in Iceland and Sweden, in the years 2000–2012, with a stored DNA sample according to the global database WorldFengur (http://www.worldfengur.com). The first criterion was the score for pace including both horses with scores below average (5.5–7.0) and higher performing (7.5–10). The number of horses in each score for pace was in accordance with the proportion of horses getting each score annually for the last 5 years in Iceland. This provided 390 five-gaited horses with a wide distribution of scores for the other gaits. In the next step, four-gaited horses (with the score 5.0 for pace and various scores for the other gaits) were added until at least 20 horses showed each score for each gait in the range of 7.0–9.0 and as many as possible in the range of 9.5–10. This added 243 horses and enabled comparison of four- and five-gaited horses of different gait quality with respect to the *DMRT3* genotype. Horses were selected at random when possible, but in the case of few available candidates, all were selected. Finally, 34 horses were selected on the basis of the judges' comments describing quality and speed capacity of the gaits (FIZO 2012) (Table 1). As comments are not necessarily recorded for each trait for all horses, comparison with the genotype data was limited to the following variables: good beat in *walk*; good speed capacity in *trot* versus lack of speed; clear beat in *trot* and good suspension in *trot* versus four-beated *trot*; good speed capacity in *gallop* versus lack of speed; clear beat in *gallop;* good suspension in *gallop* versus lack of suspension in *gallop;* good speed capacity in *tölt* versus lack of speed; good beat in *tölt* versus trotty *tölt;* and supple *tölt* versus stiff *tölt*.

Table 1 Number of horses in the data set within scores for each gait assessed at breeding field tests for Icelandic breeding horses

Trait	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0	Total
Walk	0	9	24	47	114	140	176	105	29	2	1	647
Trot	1	0	2	19	71	95	194	172	87	25	1	667
Gallop	0	0	1	2	28	109	235	197	84	11	0	667
Canter	26	0	0	10	62	162	197	93	35	11	1	597
Tölt	0	0	2	4	21	71	182	217	112	55	3	667
Slow tölt	3	0	5	8	43	130	220	150	63	10	1	630
Pace	263	44	47	59	80	30	35	46	50	12	1	667

Description of data

The data set consisted of 667 horses, of which 360 were stallions and 307 mares. Where a horse had been scored more than once at breeding field tests, only the record where the horse obtained its highest total score for riding ability was used. Scores for *tölt* and age at first evaluation at a breeding field test were also investigated. The age ranged from 4 to 14 years, with a mean of 6.4 ± 1.7 years. The horses were born in 1986–2008 with a mean birth year 2001 ± 4.3. The total number of sires was 271, with an average of 2.5 ± 3.7 offspring per sire (range: 1–43). The data set included 404 five-gaited horses (*pace* score ≥ 5.5) and 263 four-gaited horses (*pace* score = 5.0).

SNP genotyping

Samples from the 667 horses were obtained from two repositories in Iceland and one in Sweden. DNA was extracted from nose swabs and blood using mag™ kit (AGOWA GmbH, Berlin, Germany) and Gentra Puregene Blood Core Kit (QIAGEN Inc., Venlo, Limburg, the Netherlands), respectively.

Custom TaqMan SNP Genotyping assays (Applied Biosystems, Inc., Foster City, CA, USA) were used to genotype the *DMRT3_Ser301STOP* SNP with the following primers and probes: Forward primer: 5′-CCTC TCCAGCCGCTCCT-3′; reverse primer: TCAAAGATG TGCCCGTTGGA-3′; wild-type probe: 5′-CTGCCGAA GTTCG; mutant probe: 5′-CTCTGCCTAAGTTCG-3′. rt-PCRs were carried out on a 384-well ABI PRISM 7900 HT sequence detection system (Applied Biosystems) and a 96-well Stratagene Mx3005P.

Statistical analyses

Statistical analyses were performed using SAS (SAS Institute Inc. 2009). The mean, standard deviation (SD), skewness and kurtosis were calculated to describe the variation of the gaits within the sample of 667 horses. To ascertain whether the distribution of the gaits deviated significantly from zero measure of skewness and kurtosis, the following calculations were made: Estimated skewness $>1.96\sqrt{6/n}$ for $p < 0.05$ and estimated skewness $>2.33\sqrt{6/n}$ for $p < 0.01$; and estimated kurtosis $>1.96\sqrt{24/n}$ for $p < 0.05$ and estimated kurtosis $>2.33\sqrt{24/n}$ for $p < 0.01$.

Effects of the age*sex interaction (four age classes: 4, 5, 6 and ≥7 years old horses, two sex classes: mares and stallions) and the genotype of the horse (two classes: AA and CA genotypes) on the gait traits were estimated with analysis of variance using PROC GLM (SAS Institute Inc. 2009). The following model was assumed for each gait trait:

$y_{ijn} = \mu + \text{age} - \text{sex}_i + \text{genotype}_j + \text{age} - \text{sex} * \text{genotype}_k + e_{ijn}$

where y_{ijn} is a gait trait (six traits: *walk*, *trot*, *gallop*, *canter*, *tölt and slow tölt*) for the *n*th horse, μ is the population mean, age-sex$_i$ is the combined effect of the i^{th} age–sex group (i = 1,. . .,8), genotype$_j$ is the effect of the j^{th} genotype (j = 1, 2; 1 = AA, 2 = CA), age-sex*genotype$_k$ is the effect of the interaction of k^{th} age–sex by genotype (k = 1,. . .,16) and e_{ijn} is a random ~NID (0, σ_e^2) residual effect. Because of their low number, horses with the CC genotype (n = 8) were not included in this analysis. A Student's t-test was used to ascertain whether both scores for *tölt* and age at first evaluation differed significantly between horses with AA and CA genotypes. Then a chi-square test with 1 df was performed to study whether proportions of genotypes within a subgroup of 28 horses that had received scores of 9.0–9.5 for *tölt* as 4 years old deviated significantly from the proportion of the genotypes within the whole data set.

Discriminant analysis was performed using stepwise selection to obtain a subset of the gaits to be able to discriminate between the genotype classes (AA and C-). Only gaits that were significant in the stepwise discriminant function procedure and that had partial R^2 values ≥0.01 were retained in the final model. These gaits were then included in a canonical discriminant analysis to find a linear combination of the gaits that best summarized the difference between the genotype classes (AA and C-). Mahalanobis distance between the class means was estimated. The analyses were performed using the PROC STEPDISC and PROC CANDISC (SAS Institute Inc. 2009), respectively.

Chi-square tests with 1 df were performed to study whether proportions of genotypes within groups of horses receiving certain judges' comments describing the gaits deviated significantly from the proportion of the genotypes within the whole data set.

Change in allele frequency over time

When the mode of inheritance is known, genotype probabilities at individual loci in large animal populations can be estimated from genotyped data or phenotypic data on a part of the population. For this purpose, efficient computing algorithms have been created (van Arendonk *et al.* 1989; Fernando *et al.* 1993; Janss *et al.* 1995; Kerr & Kinghorn 1996).

WorldFengur (http://www.worldfengur.com) provided a pedigree file containing 410 285 horses for this study (birth years 1860–2012) where of 83% were born after 1989. The average pedigree depth for horses born 2009–2012 was 5.1 generations (max. value 15), and the corresponding five generations pedigree completeness index (PEC) was 85% according to the method of MacCluer *et al.* (1983).

So far, only a very small proportion of the population of Icelandic horses has been genotyped for the *DMRT3* mutation (706 genotyped horses were available for this study). However, recording of *pace* scores in breeding field tests may provide approximate information on the conditional probabilities of the genotypes for a larger number of horses. As a starting point, *pace* scores were extracted from the breeding field test records kept in WorldFengur based on the following conditions: a) *pace* score ≥6.0 (indication of a carrier of the A-allele), b) *pace* score = 5.0 (no *pace* shown) and *trot* score ≥7.0 (initial indication of a C-allele carrier). Data were excluded for horses receiving 5.5 for *pace* and for horses with no *pace* shown in combination with limited or bad *trot*. In total, 55 073 records on 33 036 horses fulfilled the required conditions. The highest *pace* score for horses with repeated observations was selected, and one record per horse was used.

Six phenotypic classes were formed based on the information content of the genotype data and the phenotypic data (first two columns in Table 2). The genotype data consisted of 521 AA, 177 CA and eight CC horses. The phenotypic *pace* scores ≥7.0 were taken to indicate the AA genotype (score 1), and *pace* scores 5.0 were used as a preliminary indication of the CA or CC (C-) genotype (score 4), while *pace* scores 6.0–6.5 were assumed to exclude the CC genotype and leave scope for CA or AA genotypes (score 5).

Table 2 Phenotypic scores and corresponding possible genotypes for Icelandic horses included in the pedigree list. The distribution of the scores is shown before and after G-E updating, for the 410 285 Icelandic horses included in the pedigree list

Phenotypic scores	Genotypes	Initial scores		G–E updated scores	
		N	%	N	%
1	AA	17 284	4.21	67 019	16.33
2	CA	176	0.04	7910	1.93
3	CC	8	0.002	8	0.002
4	C-	8857	2.16	4661	1.14
5	A-	6620	1.61	160 633	39.15
9 (no score)	–	377 348	91.97	170 054	41.45

The present genotype data and earlier results (Andersson *et al.* 2012) have shown that horses with *pace* scores ≥7.0 are almost certainly of genotype AA, while a large part (>30%) of the horses receiving *pace* score of 5.0 (shown as four-gaited horse) are also AA although pace was not presented, for various reasons. By use of pedigree data and the laws of Mendelian inheritance, the preliminary phenotypic scores can be updated and improved. It is feasible to use the Genotype Elimination (G–E) algorithm of Lange (1997) to improve the phenotypic score data by creating a legal data set compatible with the pedigree and Mendelian models (Table 2). The G-E algorithm is an iterative procedure for eliminating genotypes where incompatibility is observed between any offspring–parent pairs in the pedigree list. The G-E algorithm was run repeatedly (seven times), and inconsistency was listed. Unlikely scores of offspring were adjusted to score 1 whenever both parents had genotype AA confirmed on the basis of genotype or phenotypic data. As many horses shown as four-gaiters are truly AA, this procedure of updating seems important (Andersson *et al.* 2012). The data with G-E updated scores were used as an input in the segregation analysis by the Geneprob Fortran program of Kerr & Kinghorn (1996). The Geneprob program is based on the concept of 'iterative peeling' and, as all such algorithms, which are based on probability equations, the method is sensitive to inconsistency in the data. The prior use of the G-E procedure is highly recommendable before segregation analysis in large data sets where errors in pedigree and/or data recording are inevitable.

The resulting changes in the phenotypic scores are shown in Table 2. The increase in fully and partly informative scores was from 8.03% in the initial data to 58.55% in the G–E updated scores.

The frequency of the C-allele in the founder population, p(C), was assumed to be either 0.13 as in the sample of 706 genotyped horses or 0.30, which may be a more probable value in the founder population (ca. 5 generations back) according to preliminary results. These values were used as priors in the genotype probability computations. Many horses in the population were slightly inbred (average inbreeding coefficient was 2.5%). The prior genotype probabilities for inbred animals are not exact as the algorithm in Geneprob does not account for the increased probability of inbred animals being homozygous. For animals with sufficient phenotypic or genotypic information, the effects of prior allelic frequency or inbreeding level on the posterior genotypic probabilities are negligible.

The accuracy of the genotype probability estimates was evaluated by the genotype probability index (GPI) developed by Kinghorn (1997) to indicate the information content from the segregation analysis.

Mean genotype probabilities within each year were the estimate for the genotype frequencies within each cohort, and from these, the annual development in the frequencies of the A- and C-alleles was plotted for the birth years 1980–2012. A chi-square test with 1 df was performed to evaluate whether the genotypes would conform to the Hardy–Weinberg proportions. The chi-square value for each year (cohort) was regressed on year for the period 1980–2012. These calculations included 146 763 horses with a GPI of ≥30% (Kinghorn 1997).

Results

Effect of DMRT3 *genotype on gait traits*

The majority of the 667 horses genotyped for the *DMRT3_Ser301STOP* mutation, or 509 (76.3%), were homozygous for the A-allele (AA) and 150 (22.5%) were heterozygous (CA) while only 8 (1.2%) were found homozygous for the wild type (CC). Accordingly, the frequency of the A-allele was 0.88 and of the C-allele 0.12 in this data set and the genotypes conform to the Hardy–Weinberg proportions. Among the four-gaited horses, 118 of 263 (45.0%) were homozygous AA, 137 (52.0%) heterozygous CA and 8 (3.0%) homozygous CC, while 391 of 404 (96.8%) five-gaited horses were homozygous AA and 13 were heterozygous CA (3.2%). The 13 five-gaited horses with the CA genotype had scores from 5.5–7.0 for *pace* with a mean score of 5.92, compared with a mean score of 7.30 for horses of the AA genotype.

The mean, range and variation of six gait traits are presented in Table 3. The distribution of the traits *walk, trot* and *canter* deviated significantly from zero measure of skewness and kurtosis.

The *DMRT3* genotype had a significant effect on all gaits except *slow tölt* (Table 4). Scores for *walk, trot, gallop* and *canter* were significantly higher among horses with the CA genotype compared with AA horses which had significantly higher scores for *tölt.*

The interaction term between the age–sex classes and genotype (two classes: AA genotype and CA genotype) proved to be non-significant for all gaits except for *tölt*. Stallions aged four and 5 years with the AA genotype had significantly higher scores for *tölt* than their contemporaries with the CA genotype. Mean scores of 4-year-old stallions with the AA and CA genotype were 8.55 and 7.90, respectively ($p < 0.01$), and mean scores of 5-year-old AA and CA stallions were 8.48 and 8.18, respectively ($p < 0.05$). Moreover, 6-year-old mares with the AA genotype had significantly higher scores for *tölt* (8.25) than 6-year-old mares with the CA genotype (7.93) ($p < 0.05$). Mean scores for *tölt* at first evaluation in breeding field tests for CA and AA horses were 8.11 (mean age: 5.5 years) and 8.15 (mean age: 5.1 years),

Table 3 The mean, range and variation of six gait traits of the 667 horses included in the data set

Trait	Mean	SD	Min	Max	Skewness	Kurtosis
Walk	7.66	0.73	6.00	9.50	−0.25*	−0.37
Trot	8.09	0.69	6.00	10.00	−0.18	−0.39*
Gallop	8.16	0.55	6.50	9.50	−0.14	0.07
Canter	7.89	0.60	6.50	10.00	0.29*	0.11
Tölt	8.36	0.64	6.00	10.00	−0.19	0.29
Slow tölt	8.05	0.59	6.00	10.00	−0.04	−0.13

Levels of significance: *$p < 0.05$.

Table 4 Results of analysis of variance for the effect of *DMRT3* genotype on gait traits (667 horses). Least square means of six gait traits of homozygous mutant (AA) and heterozygous (CA) horses. The p-values indicate where there is significant difference between least square means

Trait	Number of AA	Number of CA	AA	CA	p-value
Walk	502	143	7.52	7.71	*
Trot	509	150	7.99	8.24	***
Gallop	509	150	8.08	8.36	***
Canter	474	119	7.61	8.32	***
Tölt	509	150	8.39	8.26	*
Slow tölt	488	136	8.01	8.04	NS

Levels of significance: *$p < 0.05$; ***$p < 0.001$.

respectively. CA horses were significantly older at first evaluation than AA horses, while the difference in mean score for *tölt* was not significantly different. Further, it was shown that significantly more horses had the AA genotype (93%) compared with the CA genotype (7%) within a subgroup of 28 horses that had received 9.0 or higher for *tölt* at the age of 4 years.

The selection procedure of STEPDISC was used to select the subset of the gaits that best reveals the difference between the genotype classes. All gaits were selected in the final model, and multivariate tests (Wilks' lambda and Pillai's trace) indicated highly significant ($p < 0.001$) differences between horses with the AA and C- genotypes. However, *pace* followed by *canter*, *tölt*, *gallop* and *trot* had, according to their R^2 and F-values, more discriminant power than *walk* and *slow tölt* (results not shown), so the latter were removed from the final model. In the canonical discriminant analysis, the canonical coefficients generated were significant ($p < 0.001$). The adjusted canonical correlation between the resulting discriminant function and the classification variable of the AA or C-genotype was 0.58. The structure of the discriminant function is shown in Table 5. The traits with the highest absolute canonical coefficients or loadings contribute the most to the divergence between genotype classes. *Canter* had the highest positive coefficient, followed by *gallop* and *trot*; high scores for these traits indicated a C- genotype. *Pace* and *tölt* had negative coefficients, with *pace* having a higher loading value; high scores for these traits indicated an AA genotype. The mid-point between the group centroid scores, which may be used as a cutting point to assign a previously unclassified horse to a genotype group, was 0.52; a horse with a value >0.52 would be classified as a C- horse and a horse with a value <0.52 would be classified as an AA horse. The Mahalanobis distance (D^2) was 3.23 and showed a significant difference between the genotype classes, indicating that horses would be correctly classified in 82% of all cases.

Table 5 Total canonical structure of the discriminating function separating horses of the AA and CA genotypes

Trait	Coefficient
Pace	−0.79
Canter	0.59
Gallop	0.40
Trot	0.27
Tölt	−0.11
F-value	62.72
p-value	***

Levels of significance: ***p < 0.001.

The proportions of the AA and CA genotypes differed significantly within groups of horses receiving judges' comments describing beat in *trot, gallop* and *tölt*; suspension in *trot* and *gallop*; and speed capacity and suppleness in *tölt*. Horses with the CA genotype more often had good suspension in *trot* and *gallop*, better beat in *gallop* and were less likely to be four-beated in *trot* while AA horses were more likely to be supple in *tölt* and to possess good speed capacity in *tölt* (Table 6).

Change in allele frequency over time

The results of the segregation analyses with the two different prior allele frequencies (p(C) = 0.13 and p(C) = 0.30) were compared in terms of information content (Table 7). The true allele frequency for the C-allele is probably closer to 0.3 in the founder population (see Figure 1). The frequency of accurately estimated genotypes was slightly higher, and therefore, only the results from the analysis with p(C) = 0.3 will be presented and discussed further.

The increase in exactly evaluated genotypes in the segregation analysis compared with the G-E procedure is shown in Table 8. The data included eight

Table 6 The proportion of genotypes within groups of horses receiving certain judges' comments for the gaits. The p-values indicate where the proportions deviate significantly from the expected proportions of 0.76 for the homozygous mutant genotype (AA) and 0.24 for the heterozygous genotype (CA) according to a chi-square test with 1 degree of freedom

Trait	Number	AA	C/-	p-value
Walk				
Clear beat	132	0.73	0.27	NS
Trot				
Good speed capacity	123	0.79	0.21	NS
Lack of speed capacity	88	0.83	0.17	NS
Clear beat	131	0.74	0.26	NS
Four-beated	64	0.91	0.09	*
Good suspension	92	0.45	0.56	***
Gallop				
Good speed capacity	166	0.79	0.21	NS
Lack of speed capacity	35	0.83	0.17	NS
Clear beat	65	0.6	0.4	*
Good suspension	77	0.42	0.58	***
Lack of suspension	71	0.96	0.04	***
Tölt				
Good speed capacity	222	0.83	0.17	*
Lack of speed capacity	38	0.53	0.47	***
Clear beat	257	0.78	0.22	NS
Trotty beat	27	0.19	0.81	***
Supple	99	0.87	0.13	*
Stiff	33	0.82	0.18	NS

Levels of significance: *p < 0.05; ***p < 0.001.

Table 7 Illustration of information content in the studied data for estimation of genotype probabilities. The genotype probabilities are derived from segregation analyses with two different prior allele frequencies (p(C) = 0.13 and p(C) = 0.30)

	P(C) = 0.13		P(C) = 0.30	
GPI	N	%	N	%
100	75 059	18.29	75 232	18.34
90	84 214	20.53	85 106	20.74
80	94 623	23.06	98 536	24.02
70	109 773	26.76	118 732	28.94
60	119 944	29.23	135 489	33.38
50	134 662	32.82	161 567	39.38
40	183 269	44.67	199 211	48.55
30	254 340	61.99	226 410	55.18

GPI, Genotype Probability Index.

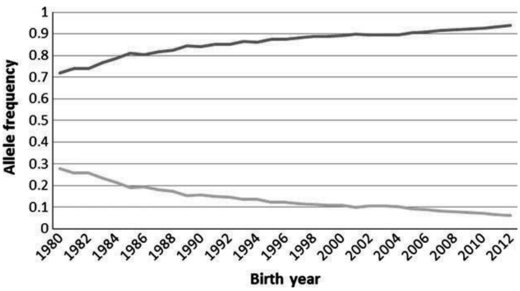

Figure 1 Frequencies of the A- and C-alleles in *DMRT3* in the Icelandic horse population from 1980–2012; the red line refers to the A-allele, and the green line refers to the C-allele.

Table 8 Number of exactly estimated genotypes in the segregation analysis when prior p(C) = 0.3

Genotypes	N	%	Increment compared with G–E updated scores
AA	67 273	16.40	254
CA	7950	1.94	40
CC	9	–	1

genotyped individuals with the CC genotype, and only one horse with exactly confirmed CC genotype was additionally revealed in the segregation analysis. Then, 24 horses were found to have estimated genotype probability >0.85 for the CC genotype.

The trend from 1980 to 2012 in the frequency of the alleles A and C was estimated in the whole population through calculation of genotype probabilities. The frequency of the A-allele was estimated to be 0.72 in 1980 and 0.94 in 2012 (Figure 1).

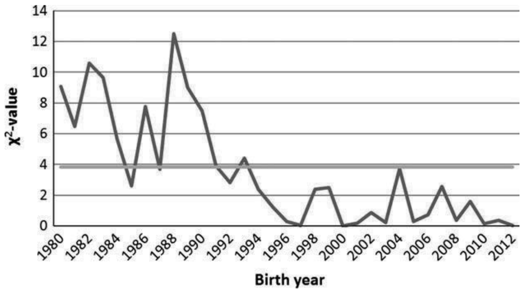

Figure 2 Development of chi-square values over time, testing Hardy–Weinberg equilibrium of *DMRT3* genotypes in the Icelandic horse population, with indicated 0.05 significance level for one degree of freedom (green line).

The chi-square test was used to evaluate whether the genotypes reflected Hardy–Weinberg proportions. This was performed for each birth year from 1980 to 2012, and the chi-square value regressed on birth year (Figure 2). For the years 1980–1993, the genotypes were not in Hardy–Weinberg equilibrium as the values were above 3.84, which is the 0.05 significance level for one df. In this period, the proportion of the CA genotype was higher than expected and the proportion of the homozygotes was subsequently lower. In the years 1994–2012, the genotypes were estimated to be in Hardy–Weinberg equilibrium in the population. The results showed that the proportion of the genotypes in the selected material of 667 horses conformed to the Hardy–Weinberg proportions. These findings therefore agree well with the fact that the majority of horses in the selected material are born in 1997–2005.

Discussion

The population of the multigaited Icelandic horse allows for detailed estimation of the effects of the *DMRT3* nonsense mutation (*Ser301STOP*) on gaiting ability. The assessment of the different gaits is systematic and standardized (FIZO 2012), and the population is not fixed for the mutation (Andersson *et al.* 2012). In this study, the effect of the *DMRT3* nonsense mutation on the gaiting ability of the Icelandic horse was estimated using more detailed information and a larger sample of assessed breeding horses than previously published (Andersson *et al.* 2012). The horses in the data set were selected with regard to scores and judges' comments referring to the individual gaits to include as detailed information about both gait quality and speed capacity as possible. The mean scores for the gaits (Table 3) were, however, higher in the selected material than in all Icelandic breeding horses presented for breeding assessment in a similar period (Albertsdóttir *et al.* 2008), except for *walk* and *pace*. No single sire is believed to have a great impact on the results as the average number of offspring per sire is low and it is assumed that the data set reflects the estimated proportion of genotypes in the population. The rider has been shown to have a significant effect on gait quality (Albertsdóttir *et al.* 2007). The rider effect was, however, not included in the model where the genotype effect on the gaits was estimated because of obvious risk of confounding effects of the rider and genotype, as 74% of riders rode only one or two horses. Kerr and Kinghorn's method (1996) of calculating genotype probabilities facilitated the estimation of the development in the frequency of the A- and C-alleles, which shows how breeding decisions have shaped the distribution of *DMRT3* genotypes over time.

Effect of the DMRT3 nonsense mutation on gait traits

This study confirmed favourable effects of the *DMRT3* nonsense mutation on the lateral gaits *tölt* and *pace*. Almost all horses with a *pace* score of 5.5 or higher were homozygous for the *DMRT3* nonsense mutation, confirming that the AA genotype is a prerequisite for the ability to pace. The AA genotype is, however, not sufficient for the ability to perform *pace* as 45% of horses classified as four-gaited were homozygous mutants. This high proportion of AA horses presented as four-gaited (without *pace*) could thus be influenced by other genetic and environmental factors. Presenting all gaits at breeding field tests will give the possibility of highest total score and is therefore the main goal. However, the score for *tölt* is the most valuable trait for the marketing price of the horse, so *tölt* has the highest weight in the total score. Presenting four gaits is an alternative, preferable for horses that do not have outstanding performance in *pace*. Training of *tölt* receives the highest priority and it is well known that pace training can in some instances impair the *tölt* quality. Therefore, many horses are ridden as four-gaiters even if they could perform *pace* up to a certain level (Árnason & Sigurdsson 2004). The presence of few CA horses receiving a score of 5.5 or higher for *pace* (3.2% of the five-gaited horses), which all received scores below average for *pace*, is most likely a phenotypic misclassification or in some instances presumably resulting from training, conformation or other factors that can facilitate CA horses to perform low quality pace. *Tölt* and *pace* are very similar gaits, both being in fact four-beat, lateral gaits (Wilson *et al.* 1998), and phenotypic misclassification is therefore not unexpected. The main features that separate them is the shorter time between ground contact of lateral legs in *pace* than *tölt* and a moment of suspension in *pace*, which should be non-existing in *tölt* (Zips *et al.* 2001).

The results clearly showed a positive effect of the AA genotype on the *tölt* ability. This seems to depend both on superior speed capacity and suppleness of the AA horses compared with the CA horses. Speed capacity and suppleness greatly impact the scoring for *tölt* (FIZO 2012). The significant interaction between genotype and the age–sex classes in the analysis of variance indicates that AA horses have more natural ability to tölt. CA horses were also significantly older when presented at breeding field tests for the first time. This could indicate that they need longer training than AA horses to develop an acceptable *tölt* capacity, as the quality of *tölt* is one of the main criteria for the preselection of horses to the breeding field tests (Albertsdóttir *et al.* 2011). Moreover, AA horses are overrepresented in the group of 28 horses in the data set that had received a score of 9.0 or 9.5 for *tölt* at the age of four. Heterozygous horses had significantly higher scores for the basic gaits *walk, trot, gallop* and *canter*. A previous study (Andersson *et al.* 2012) has shown that Icelandic horses with the CA genotype had significantly higher scores for *trot* compared with homozygous mutant horses. This was confirmed in the current study and further related to correct beat and suspension. It was also revealed that CA horses had significantly higher scores for *gallop* and *canter* compared with AA horses, possessing more often correct beat and suspension in *canter/gallop*. Correct beat in *canter* (a pure three-beat) depends on synchronized movement of diagonal legs in much the same way as in *trot* (Clayton 2004). These results indicate that the AA genotype reinforces the coordination of ipsilateral legs, with the subsequent negative effect on the synchronized movement of diagonal legs. This agrees well with previous suggestions that DMRT3 neurons play a critical role in left/right coordination, as well as in coordinating the movement of fore- and hind limbs (Andersson *et al.* 2012). The negative effect of the AA genotype on beat and suspension in *trot* as well as in *canter/gallop* has probably the same cause, suggesting a negative effect of the AA genotype on the synchronized movements of diagonal legs. The genotype effect on scores for canter is strong (Table 4), but high scores for canter demand correct beat and suspension (FIZO 2012). This is further supported by the higher proportion of horses with the CA genotype among horses that received the judges' comment *trotty tölt,* which involves too much association of diagonal legs in *tölt*.

Standardbred trotters with the AA genotype have been reported to have significantly higher breeding values for racing performance compared with the CA genotype (Andersson *et al.* 2012). It was suggested that the AA genotype promotes speed capacity at *trot.* This was not supported in the current study probably because riders at breeding field tests for Icelandic horses are not always riding them to their limit in speed in *trot* to maintain correct beat, as correct beat counts more than high speed in the scoring for *trot* (FIZO 2012). It has been suggested that the transition from *trot* to *gallop* is triggered when musculoskeletal forces reach a critical level and that peak forces are reduced at a certain speed by the transition from *trot* to *gallop,* as *gallop* is a more compliant gait with the sequential ground contact of limbs (Farley & Taylor 1991). This critical level could be avoided at high speed in *trot* by dissociating diagonal legs (become four-beated) and therefore placing the legs more sequentially on the ground. It has, indeed, been shown that the magnitude of diagonal dissociation increases with speed (Drevemo *et al.* 1980). Therefore, the superiority of AA horses in *trot* racing could be explained by their 'ability' to be four-beated in *trot* as shown in the current study. That could be an advantage when high speed in *trot* is required but a disadvantage when qualities such as correct beat and suspension are required.

The results of the canonical discriminant analysis supported the findings of the analysis of variance for the effect of the *DMRT3* genotypes on the gaits. Based on scores for *trot*, *canter*, *gallop*, *tölt* and *pace*, it was possible to discriminate between AA and CA horses with high confidence. *Pace* had the highest negative loading and, along with a high score for *tölt*, suggested an AA genotype. High scores for *trot canter* and *gallop* suggested a CA genotype, with *canter* having the greatest discriminating power of the basic gaits.

Change in allele frequency over time

The change in the frequency of the A- and C-alleles indicated a selection in favour of the A-allele in the Icelandic horse population over the last decades. This result must be interpreted in the light of the effect of the AA genotype on *tölt* and its crucial role for the ability to pace. Since the definition of the official breeding objective for the Icelandic horse in 1950 and until now, an excellent five-gaited horse has been the main aim (Hugason 1994). This entailed a heavy emphasis for many years on the selection of Icelandic breeding horses with good capacity for both *tölt* and *pace*. Breeding value estimations for Icelandic horses are based on breeding field test scores, where *tölt* has the highest weight and *pace* a relatively high weight. In addition, both traits have high genetic variation compared with other assessed traits, especially *pace* (Albertsdóttir *et al.* 2008). The quality of horses with respect to these traits therefore greatly impacts their ranking, and breeders have to a greater extent based their selection on breeding values since 1984 (Árnason & Van Vleck 2001). The observed trend in the genotype frequencies implies that the C-allele may be lost in the Icelandic horse population around year 2030. In the light of the favourable effect the C-allele seems to have on the basic gaits, the breeding goal for the Icelandic horse should perhaps be redefined. Competitions for four-gaited horses, where the basic gaits and *tölt* have equal weights, have become more popular resulting in high market value of high-class four-gaited horses (Albertsdóttir *et al.* 2007). The current study indicates how probability calculations can be used to estimate the genotype of an individual using genotype and phenotypic information combined with prior knowledge about genotype effect. The results demonstrate the strength of genomic methods in monitoring the effect of breeding decisions on genetic variability.

In the years 1980–1993, the *DMRT3* genotypes were not in Hardy–Weinberg equilibrium. The observation of a higher proportion of CA horses than expected from the gene frequencies suggests compensatory mating, where the mating of two four-gaited horses has probably been avoided over these years. The reason for possible reduction in compensatory mating according to *DMRT3* genotypes and a consequent Hardy–Weinberg equilibrium in the population in the years 1994–2012 can probably be explained by decreased selection intensity on the mares' side due to expansion in population size (Sigurdardóttir 2011), combined with growing popularity of four-gaited horses among which the AA genotype is more common.

Conclusions

Homozygosity for the *DMRT3* nonsense mutation is permissive for *pace* and has a major effect on the quality of *tölt*, *trot* and *canter/gallop,* and speed capacity in *tölt.* Selective breeding for lateral gaits in the Icelandic horse population has altered the frequency of *DMRT3* genotypes with a predicted loss of the C-allele in relatively few years. The results have practical implications for breeding and training of Icelandic horses and other gaited horse breeds.

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Competing interests

Lisa S. Andersson and Gabriella Lindgren are co-inventors on a patent application concerning commercial testing of the *DMRT3* mutation. Other authors do not have any actual or potential competing interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Attritbutes assessed in the scoring of gait traits in breeding field tests for Icelandic horses and their weight in the total score.

III

Association of conformation and riding ability in Icelandic horses

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Abstract

The official breeding goal for the Icelandic horse promotes five-gaited horses with a functional and aesthetic conformation. The objectives of the present study were to assess the phenotypic and genetic relationship between standard conformational measurements and scores for riding ability. Further, to investigate if more detailed (3-D) morphometric measurements could discriminate between high-class and low-class horses based on scores for each gait. The data comprised records from standard conformational measurements and scores for the different gaits and the total score for riding ability of all assessed breeding horses in Iceland in 2000-2013 (10,091 horses). Further, records from a subpopulation of 98 haphazardly selected breeding horses that were subject to detailed quantification of the conformation in 3-D and genotyped with respect to *DMRT3* genotype, were included in the study. Most of the standard measurements had a significant curvilinear relationship with the studied riding ability traits. They had generally high estimated heritability but weak or moderate genetic correlation with the total score of riding ability. Proportions in the top line of the horse describing the height of the horse at front compared to hind were found to be most important for the riding ability, revealing the advantage of an *uphill* conformation. Their estimated heritability and genetic correlation with total score for riding ability designate them as important indicators for performance. Certain lengths, proportions and angles between bones in the fore- and hind limbs also had a significant effect on scores for some gaits. These results can improve the assessment of the conformation and consequently the riding ability of the Icelandic horse.

Keywords: Gaiting ability, morphometric measurements, objective quantification, *DMRT3*.

Introduction

The Icelandic horse is bred for leisure riding and sport competitions. The international breeding goal promotes a functional, sound and aesthetically appealing conformation and the ability to perform five gaits; walk, trot, canter/gallop, *tölt* and pace. Systematic evaluations of Icelandic breeding horses are performed annually at breeding field tests in more than 10 countries in Europe and North America. The horses are subjectively assessed for 15 composite traits of conformation and riding ability (FIZO, 2014). As an aid for the subjective evaluation of the conformation, standard conformational measurements are performed on all horses. Each trait is assessed on a scale from 5 (not presented) to 10 (best). The assessment of riding abilities includes scoring of the five gaits under rider with respect to qualities such as correct beat, suppleness, stride length, leg action, speed capacity, collection and lightness (Supplementary table). The total score for riding abilities is a combination of the weighted scores for all gaits together with the traits spirit and form under rider. Scores are also given for slow *tölt* and canter, which influence the scoring for *tölt* and gallop, respectively, although they are not weighed into the total score (FIZO, 2014).

The estimated heritability of the subjectively assessed traits of conformation and riding ability in Icelandic horses has been reported to range between 0.22 - 0.46 and 0.20 - 0.63, respectively with most of them having a positive genetic correlation (Albertsdóttir *et al*. 2008).

In a number of studies the conformation of horses has been related to performance where various conformational aspects are considered favourable in certain disciplines. These studies have involved linear type conformation traits (Rustin et al., 2009), measurements using photogrammetric methods (Holmström et al., 1990) and images provided by video records (Back et al., 1996; Crevier-Denoix et al., 2006; Weller et al., 2006a). The method of Crevier-Denoix et al. (2006) has been used to quantify the conformation of the Selle Francais horse and the Icelandic horse and found to produce repeatable data (Kristjansson et al., 2013). Reynisdóttir (2001) quantified the conformation of Icelandic horses in a pilot study, using a photogrammetric method and reported significant relationships of some conformational parameters with riding ability. These findings suggested the need for further investigation, preferably about the relationship between objectively obtained morphometric parameters and the different traits of riding ability in the Icelandic horse. It has been reported that the heritability of measurements is generally higher than for subjectively scored composite traits (Dolvik and Klemetsdal, 1999; Suontama et al., 2009) and results support the practice of indirect performance selection through selection for functional conformation (Schröder et al., 2010).

A nonsense mutation in *DMRT3* (*DMRT3_Ser301STOP),* referred to as the ´Gait keeper´ mutation, has been reported to have major impact on the gaiting ability of Icelandic horses as it was found to be permissive for the ability to perform the lateral gaits *tölt* and pace (Andersson et al., 2012). Homozygosity for the mutation is required although not sufficient for the ability to pace while it has a negative effect on scores for the basic gaits (Andersson et al., 2012; Kristjansson et al., 2014).

The objectives of the present study were to assess the phenotypic and genetic relationship between the standard conformational measurements and scores for riding ability. Further, to investigate if more detailed (3-D) morphometric measurements could discriminate between high-class and low-class horses, based on scores for the different gaits.

Material and methods

Collection of data

Records from standard conformational measurements and assessments of the riding ability were obtained from the global database WorldFengur (www.worldfengur.com) for all horses presented at breeding field tests in Iceland in the years 2000 – 2013. The material included in total 20,527 records on 10,091 horses (2,089 stallions and 8,002 mares). The age range was 4 to 18 years, with a mean of 6.0 years. The horses were sired by 1,531 sires, with 1-410 offspring each. The standard (direct) conformational measurements included stick measurements: M1 to M5; large calliper measurements: M6 to M8; small calliper measurement: M9; tape measurements: M10 and M11 (Table 1). Complete measurements were only performed on the stallions while only 6 measurements were available for the mares (M1, M3, M4, M5, M9 and M10). Calculated conformational traits included: the difference between height at withers and height at back (M1-M2) and the difference between height at withers and height at croup (M1-M3), both referred to as *height at front*; the difference between height at croup and height at back (M3-M2), referred to as *back incline*; the difference between the length of the horse and height at withers (M5-M1) and the difference between length of the horse and height at croup (M5-M3), both referred to as *format of the horse* and the difference between the hip measurements (M7-M8), referred to as *form of the croup*.

A subpopulation of 98 horses (25 stallions and 73 mares) was haphazardly selected at breeding field tests in Iceland in the years 2008-2010 for more detailed morphometric measurements. The age of the horses within the subpopulation ranged from 4 to 10 years, with a mean of 6.0 years, sired by 66 sires, with 1-7 offspring each. The subpopulation included 72 previously described horses (Kristjansson et al., 2013) and additionally 26 horses that were included to provide a material with a wider distribution of scores for the different gaits. The conformation of these horses was objectively quantified using a three-dimensional video morphometric method developed by Crevier-Denoix et al. (2006). The method has been described in detail by Kristjansson et al. (2013). A total of 28 anatomical landmarks were tracked on each horse; for the head and neck, front- and hind part of the horse (see Figures 1 -

3 for the location of each landmark) allowing automatic calculation of 400 morphometric parameters. The calculations included heights of certain anatomical landmarks, lengths and widths of segments, joint angles, angles of certain body parts with respect to the horizontal and vertical (all these angles were real, calculated in 3-D). Furthermore, calculated conformational traits were derived from these basic traits, such as heights, lengths and widths reported as a proportion of the height at withers or as a proportion of other measurements (such as: height of base of neck/height at croup), joint angles projected in the frontal plane (corresponding to the evaluation of body parts from front or behind) or differences of various measurements. The 98 horses were genotyped according to the ‘Gait keeper’ mutation (Anderson et al., 2012) and to exclude genotype effect, only horses that were homozygous for the mutation (AA) were divided into *low-class* and *high-class* groups containing at least 20 horses with scores of 6.5-7.0 (low-class) or 9.0-9.5 (high-class) for each of the gaits: walk, trot, canter, gallop, *tölt*, slow *tölt* and pace. An additional comparison was made for horses with a score of 5.0 for pace that were compared with horses scoring 8.0-9.5 for that gait.

<<Figures 1 – 3>>

Statistical analyses

Statistical analyses were mainly performed using the SAS package (SAS, 2009). The mean, standard deviation (SD), skewness and kurtosis were calculated to describe the variation of the standard conformational measurements and the gait traits (Tables 1 and 2). To ascertain whether the distribution of the traits deviated significantly from zero measure of skewness and kurtosis the following calculations were made: Estimated skewness $> 1.96\sqrt{6/n}$ for $p<0.05$ and estimated skewness $> 2.33\sqrt{6/n}$ for $p<0.01$; and estimated kurtosis $> 1.96\sqrt{24/n}$ for $p<0.05$ and estimated kurtosis $> 2.33\sqrt{24/n}$ for $p<0.01$.

Effects of the age_sex subclasses accounting for the interaction between age and sex (four age classes: 4, 5, 6 and ≥7 years old horses*two sex classes: mares and stallions), year of assessment (14 years: 2000-2013) and a regression effect of each standard direct conformational measurement (M1 to M11) and six additional calculated measurements on the riding ability traits was estimated with analysis of variance using PROC GLM (SAS, 2009). The following model was assumed for each gait and total score for riding ability:

$$y_{ijn} = \mu + \text{age\_sex}_i + \text{year}_j + \beta \text{ measurement}_k + e_{ijn}$$

where y_{ijn} is a riding ability trait (*total score for riding ability* and scores for *walk, trot, gallop, canter, tölt, slow tölt and pace*) for the *n*th horse, μ is the population mean, age_sex_i is the fixed effect of the *i*th age_sex (i=1,…,8), year_j is the fixed effect of the *j*th year (j=1,…,14), β is the regression effect of the *k*th conformational measurement (k =*1,...,17*, see Table 1) and e_{ijn} is a random ~ND(0, σ^2_e) residual effect. The linearity of the effect of the conformational measurements on the riding ability traits was also tested by including a quadratic term as a regression parameter in the model.

The DMU package (Jensen and Madsen, 2010) was used to estimate genetic parameters. Variance and covariance components were estimated with bivariate models using the average information (AI) algorithm for restricted maximum likelihood, and the asymptotic standard errors of (co)variance components were computed from the inverse of the AI matrix. For the standard conformational measurements, calculated measurements and total score of riding ability the following model was used:

$$y_{ijm} = \text{year}_i + \text{birth-year\_sex}_j + \text{animal}_m + \text{pe}_m + e_{ijm}$$

where y_{ijm} are conformational measurements or total scores of riding ability for the *m*th horse, year_i is the fixed effect of assessment year of the *i*th year (i = 2000,...,2013), birth-year_sex_j is the fixed effect of the birth year by sex subclass of the *j*th birth-year_sex (j = 1,...,8), animal_m is the random additive genetic effect of the *m*th horse ~ND(0, $\mathbf{A}\sigma^2_a$), **A** being the numerator relationship matrix among horses, pe_m is the permanent environmental effect of the *m*th horse ~ND(0, $I\sigma^2_{pe}$) and e_{ijm} is the random residual effect ~ND(0, $\mathbf{I}\sigma^2_e$).

The 400 original 3-D morphometrical parameters were subjected to a principal component analysis using ones as prior communality estimates. The principal axis method was used to extract the principal components (PCs), followed by a varimax (orthogonal) rotation. The first 22 components displayed eigenvalues greater than 1.0 and they were retained for rotation. Combined, components 1 to 22 accounted for 90% of the total variance. The PCs summarised separate groups of common 3-D measurements, i.e. lengths, widths, angles and proportions within the conformation. For interpreting the rotated factor pattern, a morphometric measurement was said to load on a given component if the factor loading was 0.40 or greater for that component and less than 0.40 for the others. Using these criteria, the morphometric measurements were chosen for each factor. The PROC FACTOR (SAS. 2009), which was used for the principal components analysis, also produced the components as standardized scores (linear combinations of the observed 3-D morphometric measurements) for the 22 retained components. These scores were used in further analyses where the 3-D morphometric measurements were related to gait traits.

Discriminant analysis was performed by using stepwise selection to obtain a subset of the 22 PCs that discriminated between the low-class and high-class groups with respect to each gait. Only components that were significant in the stepwise discriminant function procedure and that had partial R^2 values ≥ 0.01 were retained in the final model. The 3-D morphometric measurements that loaded on the selected PCs were then submitted to canonical discriminant analysis to find a linear combination of the 3-D measurements that best summarizes the difference between the low-class and high-class groups within the subpopulation of the 98 horses. Mahalanobis distance (D^2) between the group means was estimated. The analyses were performed using the PROC STEPDISC and PROC CANDISC (SAS, 2009), respectively. For comparison, regression models were also developed for each riding ability trait using stepwise selection and based on the 22 retained components. Information on all the 98 horses was used for this analysis applying the PROC REG (SAS, 2009).

Results

Effect of standard conformational measurements on the scores for riding ability

The mean, range and variation of the standard conformational measurements and traits of riding ability are presented in Table 1 and 2. Although the majority of the traits deviated significantly from zero measure of skewness and kurtosis, transformation of the data was not considered necessary. The fixed effects of age_sex and year of assessment were significant for all the traits of riding ability ($p<0.001$). The phenotypic regression effect of the conformational measurements on the total score for riding ability was significant for all measurements, except for height of back (M2) and circumference of the carpus and metacarpus (Table 3) and generally, a curvilinear relationship was revealed (Figure 4). The difference between the optimum for the measurements (giving the highest score for riding ability) and the respective mean values for the material is demonstrated in Tables 1 and 3. The difference between the height at the withers and height at the croup had an optimum of 6.2 cm, which was around 2 times higher than the mean value. The rather low optimum for back incline (5.4 cm) revealed that a less inclined back is more ideal for performance than a sway back or a back with a downhill inclination. The optimum difference between height at withers and height at back of 11.7 cm demonstrated the advantage of high withers.

The phenotypic regression effects of the measurements on the score for each gait were also studied (data not shown). The results were similar as for the total score of riding ability, although some discrepancies were found. A difference was revealed in the effect of the format of the horse (M5-M1), where the optimum for *tölt*, trot and pace was -4.3, 3.3 and 2.6 cm, respectively. Regarding the *back incline* (M3-M2) the quadratic term was not significant for canter and pace where a linear negative relationship was identified. Moreover, the height measurements (M1-M3) did not have a significant effect on scores for pace, nor the measurements M6-M9.

Heritability of the standard conformational measurements

Estimates of heritability of the standard conformational measurements were moderately high to high (Table 1). The highest estimates were obtained for width of metacarpus and height at withers and the lowest for width of hips (M8) and circumference of metacarpus. The heritability of the calculated measurements was markedly lower compared to the standard measurements. The genetic correlation of the standard and calculated conformational measurements with total score for riding ability was in the range of -0.25 – 0.26.

<<Tables 1 – 3>>

<<Figure 4>>

Effect of 3-D morphometric measurements on riding ability

The selection procedure of STEPDISC was used to obtain a subset of the 3-D morphometrical parameters that best revealed the difference between groups of low-class and high-class horses within each gait trait. Highly significant (P<0.001) differences were found between horses in the two groups within all tested traits of the riding ability, applying multivariable tests (Wilks´ lambda and Pillai´s trace). The canonical coefficients generated in the canonical discriminant analysis were significant (P<0.001). The adjusted canonical correlation between the resulting discriminant function and the classification variable of low- and high-class horses was 0.84, 0.74, 0.64, 0.69, 0.70, 0.48 and 0.69 for walk, trot, gallop, canter, *tölt*, slow *tölt* and pace, respectively. The structure of the discriminant function for each gait is shown in Tables 4-11 as well as the mean and SD within the comparison groups. The measurements with the highest absolute canonical coefficients or loadings contributed the most to the divergence between low-class and high-class horses. The Mahalanobis distance (D^2) showed significant difference between low-class and high-class horses with respect to selected 3-D measurements, indicating that horses would be correctly classified in 91%-98% of all cases within the gaits.

Conformational parameters that described the height at front compared to hind (such as height at withers, base of withers and base of neck compared to height at croup) discriminated between high-class and low-class horses based on scores for all the gaits. The higher the horse was in front compared to the hind the more likely it was to be included in the high-class group within each gait. Height at withers was one of the important measurements separating high-class and low-class in trot, *tölt* and gallop. The length and form of the croup discriminated between the groups in trot, *tölt* and pace. Horses scoring 8.0 or higher for pace had a more inclined and longer croup (P9->P10/P12) compared to horses with 5.0 for pace; while high-class horses for trot and *tölt* had shorter croups compared to low-class horses. High-class horses in canter and slow *tölt* had a wider pelvis (measured between the hip joint: P8-P8) than low-class horses. Lengths, proportions and joint angles within front and hind legs discriminated between high-class and low-class horses for most of the gaits. Length of the front and hind legs, measured as the height of the head of radius (A6) for the front legs and the height of the patella (P7) for the hind legs, relative to height at withers, discriminated between the groups for walk, high-class horses having longer limbs. High-class horses in trot and canter had a longer front pastern relative to the length of the metacarpus than low-class horses, while the opposite was the case for pace. Length of the tibia discriminated between high-class and low-class horses within walk, trot, gallop and *tölt*, high-class horses having longer tibia. The humerus was shorter in high-class horses in walk and trot. High-class horses in walk had a more inclined femur and smaller stifle joint compared to low-class horses. High-class horses in gallop had smaller tarsal angle in lateral view than low-class horses. The inclination of the scapula affected performance in gallop and *tölt* where high-class horses had more inclined scapula. High-class horses in *tölt* had a smaller front fetlock angle in lateral view than low-class horses. The ideal position of the hind legs (measured as the horizontal distance between the hock and tuber ischiaticum) varied between canter and pace; a more camped under hind legs was more ideal for canter, while the opposite was the case for pace. High-class horses within gallop, *tölt* and slow *tölt* had longer necks than low-class horses. The regression models that were developed for each trait of the riding ability based on the 22 retained components gave very similar results as the canonical discriminant analysis and the discrepancies were found unimportant.

<<Tables 4-11>>

Discussion

Assessment of conformation has a long tradition in horse breeding. In recent years increased emphasis has been put on functional conformation that enhances health, longevity and performance. Different methods of objective quantification of the conformation have been developed that can supply breeders with conformational parameters that can be used as indicators for better soundness and performance. The associations of conformational parameters and riding ability in the Icelandic horse have not been estimated in such detail before. In the present study both the standard conformational measurements from a large number of breeding horses were used and 3-D morphometric measurements from a limited material, enabling a more detailed description of the conformation.

The proportions in the topline of the horse describing height at front compared to hind were found important for riding ability in the Icelandic horse, revealing the advantage of high withers and an uphill conformation. Results from both the standard and 3-D conformational measurements demonstrated that high-class horses were higher at front (withers, base of withers, height at base of neck and back) compared to hind (height at croup and tuber coxae) than low-class horses. One of the attributes of riding ability that are rewarded at breeding field tests for Icelandic horses is the ability for self-carriage, collection and lightness in the front part (FIZO, 2014). The absolute and relative elevation of the forequarters in relation to the hind part has been regarded to be a prerequisite for collection (Heuschmann, 2009), including the raising of the base of the neck and the withers, rounding of the back and coiling of the loins. This entails reduction of the weight carried by the forelimbs and increase in the weight carried by the hind limbs (Rodin, 2008; Bennett, 1992). The uphill conformation of high-class horses compared to low-class horses could facilitate this. High withers have been reported to facilitate the raising of the back (Bennett, 1992). The back is reported to assist in elevation of the forequarters and to transmit forces from the hind limb to the forehand (Clayton, 2007).

The results indicated the disadvantage of a forward (downhill) inclination in the back or a sway back. A back incline which is closer to the horizontal could be better adapted for force transmission and elevation of the forehand. This was more clearly indicated in the relationship between *back incline* and canter, where a greater degree of collection is demanded than for most of the other gaits.

The optimum measurement of height at withers (M1), both for the total score for riding ability and all the different gaits, was around 145 cm; however difference in height at withers from

138 to 148 had a small effect. Significant correlations have previously been found between withers height and kinematic variables, for example a positive correlation between withers height and stride length (Sanchez et al., 2013), and a positive correlation has been identified between height at the withers and scores for gaits under rider in Swedish Warmblood horses (Holmström and Philipsson, 1993). In the current study, high-class horses in trot, gallop and *tölt* were higher at the withers compared to low-class horses. It is suggested that the positive effect of height at the withers on gaits reflects both an effect of increased leg length, which leads to increased stride length, and more elevated and/or tall withers as less effect was identified for the height at croup. Stride length is highly rewarded in the assessment of gaits at breeding field tests for Icelandic horses (FIZO, 2014). The horses with scores of 9.0-9.5 in walk, where long stride length is demanded, had longer front and hind legs as depicted by the height of head of radius and height of patella relative to height at the withers (A6/A8 and P7/P12, respectively). Further, height of the horse has been claimed to have a positive influence on the judges´ impression of the horse and tall horses therefore getting higher scores for gaits than small horses (Holmström and Back, 2013).

The results of the current study demonstrate that a square format rather than a rectangular one is beneficial for the riding ability, when the body format was studied as the difference between length and height at withers. There was, however, a discrepancy in this effect between the different gaits as a longer body format was ideal for trot and pace but not for *tölt*. Rustin et al. (2009) reported that a rectangular body format compared to a square body format had a positive effect on linear traits of walk and trot in Belgian Warmblood horses, the frame of the horse having the highest genetic correlation with stride length in trot. The optimum of -1.7 cm for total score for riding ability can be explained by the low optimum for *tölt* (-4.3) as it has the highest weight in the total score (15%). It has been shown that the body format of the Icelandic horse has changed in recent decades from a rectangular body format towards a square one (Kristjansson et al., 2013). It is likely that this change in body format has been facilitated by the strong selection for *tölt* in the same period. When the frame of the horse described by the difference between length (M5) and height at the croup (M3) was related to scores for the different gaits, less discrepancy was found. That suggests a special importance of high withers for performance in *tölt*. In the subjective scoring of conformation of Icelandic horses a long body format is rewarded and the difference between length of the horse (M5) and height at withers (M1) is used for the assessment (FIZO, 2014). This can have negative impact on the important traits height of the withers and the uphill conformation and can be avoided by using height at the croup instead of the assessment of the body format, as it is perhaps a more suitable measure of size.

Lengths of segments (bones), proportions and angles between them were found to have discriminating effect on the different gaits. High-class horses in walk had a more forward sloping femur than low-class horses and a smaller stifle joint. This agrees with Holmström and Philipsson (1993) which reported a long and forward sloping femur to be beneficial for performance, as it places the hind leg further forward under the horse and, thus, possibly facilitating the function of the hind limbs and improving the balance. The current study demonstrates that the hind legs were more camped under high-class horses than low-class horses in canter as depicted by the position of hock (P6) with respect to the tuber ischiaticum (P9). This places the hind leg closer to the center of gravity and thereby possibly providing more upward than forward propulsion (Clayton, 2013) of the hind limbs which is important for good performance in canter (FIZO, 2014).

One of the conformational parameters that discriminated between low-class and high-class horses in *tölt* was the angle of the front fetlock joint in the lateral view. The high-class horses had a smaller angle, which means a more extended fetlock joint. It has been shown that a more extended front fetlock joint results in more maximal extension which is related to good gait in the forelimb; a suppler strut and more protraction of the forelimb (Back et al., 1994; Back et al., 1996). Suppleness and stride length are rewarded in the assessment of *tölt* in the breeding field test (FIZO, 2014). High-class horses in trot and canter had a longer pastern relative to the metacarpus than low-class horses. Long pasterns have also been associated with high performing Swedish Warmblood horses in dressage (Holmström and Back, 2013).

The pelvis was wider between the hip joints (P8-P8) in high-class horses in canter and slow *tölt*. This is supported by a broader pelvis having more room for attachment of the gluteal and hamstring muscles that provide propulsion during locomotion (Clayton, 2013). Elite horses in dressage and show jumping have been found to have a more sloping shoulder (Holmström et al., 1990) and it has also been reported to correlate with longer stride length (Back et al., 1996). Horses performing high-class *tölt* and gallop in this study were found to have a more sloping shoulder (more inclined scapula).

Results have shown that homozygosity for the 'Gait keeper' mutation (A) is a prerequisite for the ability to pace, while around 45% of horses that are shown as four-gaited horses (pace score of 5.0) have been reported to be also AA (Kristjansson et al., 2014). Therefore, it was of interest to investigate whether conformational parameters could discriminate between AA horses with a pace score of 5.0 and horses that show good pacing ability. The horses that had a pace score of 8.0-9.5 had a more inclined and longer croup (both as a proportion to height at withers and length of the back) than homozygous AA horses with 5.0 for pace. This is in accordance with results from Reynisdóttir (2001). Horses with pacing ability had more straight back as depicted by the height of the base of withers to height at withers (A9/A8) and compared to height at croup (A10-A9), shorter front pasterns in relation to the metacarpus and the hind legs were more camped out (more distance between the hock and tuber ischiaticum) than in horses with 5.0 for pace. *Tölt* and pace are very similar gaits, both being in fact four-beat, lateral gaits (Wilson et al., 1998) and the main features that separate them is the shorter time between ground contact of lateral legs in pace than in *tölt* and a moment of suspension in pace, which should be non-existing in *tölt*. The group comprising horses with 5.0 for pace had a rather high mean score for *tölt* (results not shown) and therefore presumably had good speed capacity in *tölt* (FIZO, 2014). The conformational parameters that discriminated between horses with 5.0 for pace and 8.0-9.5 could include those who facilitate suspension at high speed in lateral gaits. A long and broad croup (pelvis) could provide the necessary propulsion during locomotion needed for suspension in lateral gaits as it has more room for attachment of the powerful gluteal and hamstring muscles (Payne et al., 2005). Weller et al. (2006b) showed that a large lateral coxal angle (defined as the angle between the ilium and ischium measured dorsally) was beneficial for performance in racing, possibly corresponding to a larger area for the gluteal musculature, which generate much of the motor power needed for locomotion in the hind limb. The shorter front pasterns of the horses with pacing ability could facilitate faster take off of the front limb and it therefore being in swing phase when the diagonal hind limb hits the ground and thus creating suspension in pace. When horses with pace scores of 6.5-7.0 were compared to horses with pace scores of 8.5-9.5 similar results were obtained as when horses with 5.0 for pace were compared with horses with good pacing ability. The importance of a long and inclined croup for the pacing ability was demonstrated. The high-class horses in pace also had a longer back than low-class horses and the combination with a long croup confirms the previous finding of rectangular body format to be beneficial for pace. The high-class horses in pace had a larger hip joint angle than low-class horses, explained by a more inclined pelvis and more vertical femur. The stifle joint was also larger. This could give the hind limbs more forward propulsive power.

The estimated heritability of the standard conformational measurements was in general high and comparable with earlier studies (Arnason, 1984; Dolvik and Klemetdsal, 1999; Suontama et al., 2011). The lower heritability obtained for the width of the hips between the hip joints (M8) can probably be explained by inaccuracy in measurements and greater effect of the stance of the horse on that measurement than the others. The estimated heritability of the calculated measurements ranged from 0.25 – 0.40 and was therefore lower than for the direct measurements. The genetic correlation between the standard measurements and total score for riding ability was low and in accordance with earlier results (Arnason, 1984). The width of the chest had one of the highest genetic correlations with total score for riding ability (0.21) and a rather high optimum was revealed compared to the mean value when its phenotypic relationship with total score for riding ability was estimated. The width of the chest has previously been shown to have significant genetic correlations with kinematic variables (Sanchez et al., 2013). Stronger genetic correlations were in general revealed between the calculated measurements and total score for riding ability compared to direct measurements, suggesting combinations of conformational parameters to be more valuable indicators for performance. Further, the estimated heritability of the calculated measurements describing the topline and format of the horse combined with their effect on gaiting ability designate them as the most important indicators for performance.

The conformational parameters in this study were related to subjective scores for traits of riding ability. For a more decisive estimation objectively quantified kinematic variables would be needed for the riding ability.

Conclusions

Proportions in the top line of the horse describing the height of the horse at front compared to hind were found to be most important for the riding ability revealing the advantage of an uphill conformation. Their estimated heritability and genetic correlation with riding ability designate them as important indicators for performance. Lengths, proportions and angles of many segments (bones) in fore- and hind limbs also affected scores for the different gaits and discriminated between high-class and low-class horses with high accuracy by multivariate analyses. These results can improve the assessment of the conformation and consequently the riding ability of the Icelandic horse.

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Conflict of interest

All authors declare that they have no conflicts of interest with this work.

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Figure captions:

Figure 1. Anatomical landmarks that were tracked on the reference image for the forelimb and body axis in the 3-D quantification of the conformation of the subpopulation of 98 Icelandic breeding horses.

Figure 2. Anatomical landmarks that were tracked on the reference image for the hind limb and body axis in the 3-D quantification of the conformation of the subpopulation of 98 Icelandic breeding horses.

Figure 3. Anatomical landmarks that were tracked on the reference image for the fore- and hind limb and body axis in the 3-D quantification of the conformation of the subpopulation of 98 Icelandic breeding horses.

Figure 4. Phenotypic regression effect of withers height on total score for riding ability in Icelandic breeding horses.

Tables

Table 1. Mean, range and variation of the standard conformational measurements and calculated measurements and their heritability (h^2), genetic variance (σ^2_a), permanent environmental variance (σ^2_{pe}), repeatability (t) and genetic correlation with total score for riding ability (r_g). All measurements are in cm.

Parameters:	**Mean**	**SD**	**Range**	**h^2**	**σ^2_a**	**σ^2_{pe}**	**t**	**r_{g*}**
Height at withers (M1)	139.64	3.15	126 - 153	0.67	5.92	1.69	0.87	0.06
Height at back (M2)[a]	130.85	2.89	120 - 143	0.58	4.87	2.10	0.83	-0.04
Height at croup (M3)	136.65	2.73	124 - 147	0.65	5.02	1.50	0.85	-0.05
Depth of the breast (M4)	64.17	1.72	54 - 71	0.50	1.49	0.64	0.71	-0.05
Length of the body (M5)	142.50	3.19	128 - 156	0.64	6.89	1.82	0.81	-0.06
Width of the chest (M6)	37.47	1.54	31 - 44	0.40	0.95	0.65	0.67	0.21
Width of the hips (M7)[b]	47.01	1.52	40 - 52	0.54	1.30	0.61	0.79	0.09
Width of the hips (M8)[c]	42.59	1.60	34 - 48	0.37	0.88	0.43	0.55	0.16
Width of metacarpus (M9)	6.55	0.22	6 - 8	0.85	0.14	0.03	1.00	-0.25
Circ. of carpus (M10)	28.20	1.31	23 - 33	0.53	0.37	0.17	0.77	-0.11
Circ. of metacarpus (M11)	17.95	0.81	15 - 22	0.37	0.17	0.10	0.60	-0.03
Height **at** front (M1-M2)	10.25	1.62	2 - 19	0.25	0.65	0.44	0.42	0.25
Height **at** front (M1-M3)	2.99	1.83	-5 - 12	0.27	0.73	0.56	0.47	0.26
Back incline (M3-M2)	6.08	1.52	0 - 17	0.20	0.46	0.58	0.45	-0.08
Format of the horse (M5-M1)	2.86	2.99	-9 - 15	0.40	3.17	1.59	0.60	-0.13
Format of the horse (M5-M3)	5.85	2.78	-6 - 19	0.35	2.67	1.72	0.57	0.00
Form of the croup (M7-M8)	4.42	1.46	0 - 10	0.25	0.50	0.54	0.52	-0.05

*Standard error of genetic correlation between measurements and total score of riding ability ranged from 0.04 to 0.20

[a]Height at the lowest point of the back

[b]Width of the hips between the tuber coxae

[c]Width of the hips between the hip joints

Table 2. Mean, range and variation of total score for riding ability and each gait for all shown breeding horses in 2000 – 2013 (n=20,527) and a subpopulation of 98 horses.

	All breeding horses			Sample of 98 horses		
Trait:	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**
Walk	7.52	0.74	5 - 10	7.49	0.79	5.5 - 9.5
Trot	7.76	0.67	5 - 10	7.81	0.76	5.5 - 9.5
Gallop	7.96	0.53	5 - 9.5	8.04	0.58	7 - 9.5
Canter	7.58	0.79	5 - 10	7.47	1.04	5 - 9
Tölt	8.06	0.54	5 - 10	8.15	0.65	6.5 - 9.5
Slow *tölt*	7.81	0.61	5 - 10	7.93	0.63	5 - 9
Pace	6.65	1.36	5 - 10	6.83	1.40	5 - 9.5
Total Score	7.81	0.39	5.89 - 9.25	7.93	0.43	6.90 - 8.95

Table 3. Phenotypic effect of the standard conformational measurements and calculated measurements on total score for riding ability.

	Estimate						
	Linear	P-value	Quadratic	P-value	Optimum	R^2	RMSE
M1	0.23	***	-0.0008	***	145.9	0.09	0.37
M2	0.12	NS	-0.0005	NS	-	0.11	0.38
M3	0.13	*	-0.0005	*	138.8	0.08	0.37
M4	0.28	***	-0.0022	***	63.2	0.08	0.37
M5	0.27	***	-0.0009	***	142.7	0.08	0.37
M6	0.26	*	-0.0032	*	40.2	0.11	0.37
M7	0.28	*	-0.0030	*	46.7	0.11	0.38
M8	0.24	*	-0.0030	*	44.1	0.11	0.38
M9	3.37	***	-0.26	***	6.4	0.11	0.37
M10	0.05	NS	-0.0014	NS	-	0.08	0.37
M11	0.15	NS	-0.004	NS	-	0.08	0.37
M1-M2	0.104	***	-0.0044	***	11.7	0.11	0.38
M1-M3	0.051	***	-0.0041	***	6.2	0.09	0.37
M3-M2	0.031	*	0.0028	*	5.4	0.11	0.37
M5-M1	-0.004	*	-0.0010	***	-1.7	0.08	0.37
M5-M3	0.015	***	-0.0014	***	5.4	0.08	0.37
M7-M8	-0.006	*	-0.0005	NS	-	0.11	0.38

Levels of significance: *P<0.05; **P<0.01; **P<0.001

Table 4. Total canonical structure of the discriminating function separating low-class horses (scores: 6.5-7.0) and high-class horses (scores: 9.0-9.5) within **walk** and mean and SD within the comparison groups. (Angles in lateral view). Lengths and heights in cm and angles in degrees.

	Low-class group (N = 33)		High-class group (N = 24)			All horses (n=98)
Parameters	**Mean**	**SD**	**Mean**	**SD**	**Coefficient***	**Mean**
Height of head of radius / Height at withers (A6/A8)	0.514	0.007	0.526	0.013	-0.56	0.52
Height at croup - height at base of withers (A10-A9)	2.642	2.068	0.302	2.264	0.53	1.81
Height at withers - height at croup (A8-A10)	3.875	2.207	6.117	2.281	-0.49	4.74
Lenght of tibia / Height at withers (P5->P7/P12)	0.315	0.011	0.327	0.016	-0.45	0.32
Height of patella / Height at withers (P7/P12)	0.606	0.014	0.624	0.018	-0.42	0.61
Length of humerus / height at withers (A6->A7/A8)	0.207	0.011	0.198	0.012	0.40	0.20
Length of radius (A5 -> A6)	28.741	1.277	29.562	1.244	-0.33	29.01
Stifle joint (P5-P7-P8)	120.036	5.673	117.568	6.749	0.22	119.73
Base of neck / Height at croup (T3/A10)	0.827	0.019	0.842	0.034	-0.21	0.82
Femur to the horizontal plane (P7-P8-Horiz.)	63.007	3.896	61.494	4.871	0.19	62.96
Base of neck / Height at withers (T3/A8)	0.798	0.017	0.804	0.022	0.14	0.82
Length of back (A8 -> A10)	76.951	4.091	77.130	4.941	-0.02	77.33

* Absolute canonical coefficients. The mid-point between the group centroid scores, which may be used as a cutting point to assign a previously unclassified horse to a class group, was -0.76; a horse with a value >-0.76 would be classified as a low-class horse and a horse with a value <-0.76 would be classified as a high-class horse.

Table 5. Total canonical structure of the discriminating function separating low-class horses (scores: 6.5-7.0) and high-class horses (scores: 9.0-9.5) within **trot** and mean and SD within the comparison groups. (Angles in lateral view, unless otherwise stated). Lengths and heights in cm. and angles in degrees.

	Low-class group (N = 22)		High-class group (N = 28)			All horses (n=98)
Parameters	**Mean**	**SD**	**Mean**	**SD**	**Coefficient***	**Mean**
Height at withers - height at croup (A8-A10)	3.671	2.674	5.940	1.811	-0.58	4.74
Length of croup / Height at withers (P9 -> P10) / (P12)	0.359	0.014	0.341	0.016	0.49	0.35
Height at croup - height at base of withers (A10-A9)	2.659	2.539	1.005	1.858	0.46	1.81
Pastern / Metacarpus (A2 -> A3 / A3 -> A4)	0.513	0.076	0.564	0.072	-0.42	0.54
Tarsus[a] (P3-P4 / P5-P7)	178.475	1.743	177.578	1.832	0.31	178.10
Height at withers (A8)	135.909	2.864	137.177	2.427	-0.30	136.23
Length of humerus / Height at withers (A6 -> A7) / (A8)	0.207	0.013	0.202	0.012	0.29	0.20
Length of back (Horiz. A11 -> A12)	64.479	2.952	65.784	2.933	-0.28	64.70
Metacarpus / Height at withers (A3 -> A4) / (A8)	0.142	0.007	0.139	0.008	0.20	0.14
Base of neck / Height at croup (T3/A10)	0.810	0.0159	0.824	0.26	0.07	0.82
Length of tibia (P5 -> P7)	42.735	2.338	43.001	1.762	-0.05	42.54

* Absolute canonical coefficients. The mid-point between the group centroid scores, which may be used as a cutting point to assign a previously unclassified horse to a class group, was 0.15; a horse with a value >0.15 would be classified as a low-class horse and a horse with a value <0.15 would be classified as a high-class horse.

[a] In frontal view

Table 6. Total canonical structure of the discriminating function separating low-class horses (scores: 6.5-7.0) and high-class horses (scores: 9.0-9.5) within **gallop** and mean and SD within the comparison groups. (Angles in lateral view, unless otherwise stated). Lengths and heights in cm. and angles in degrees.

	Low-class group (N = 25)		High-class group (N = 28)			All horses (n=98)
Parameters	**Mean**	**SD**	**Mean**	**SD**	**Coefficient***	**Mean**
Base of neck / Height at croup (T3/A10)	0.827	0.278	0.846	0.023	0.49	0.82
Height at withers (A8)	134.862	2.938	136.818	2.723	0.47	136.23
Length of back / Length of croup (A8 -> A10/P10 -> P9)	1.178	0.079	1.232	0.094	0.43	1.21
Base of neck / Height at withers (T3/A8)	0.802	0.018	0.813	0.019	0.42	0.82
Length of neck (T2 -> T3)	63.597	3.058	65.239	2.529	0.41	64.87
Tarsus (P3-P4 / P6-P7)	130.354	2.694	128.676	3.424	-0.38	129.18
Length of tibia (P5 -> P7)	41.841	2.339	42.904	1.921	0.35	42.54
Length of withers (A8 -> A9)	21.450	2.333	20.814	2.258	-0.20	21.07
Inclination of scapula (A7-A8-Horiz.)	56.224	3.476	55.511	3.129	-0.16	56.20
Tarsus[a] (P3-P4 / P5-P7)	178.004	2.284	178.162	1.996	0.05	178.10

* Absolute canonical coefficients. The mid-point between the group centroid scores, which may be used as a cutting point to assign a previously unclassified horse to a class group, was -0.05; a horse with a value >-0.05 would be classified as a high-class horse and a horse with a value <-0.05would be classified as a low-class horse.

[a] In frontal view

Table 7. Total canonical structure of the discriminating function separating low-class horses (scores: 6.5-7.0) and high-class horses (scores: 9.0-9.5) within **canter** and mean and SD within the comparison groups. (Angles in lateral view, unless otherwise stated). Lengths and heights in cm. and angles in degrees.

	Low-class group (N = 38)		High-class group (N = 16)			All horses (n=98)
Parameters	**Mean**	**SD**	**Mean**	**SD**	**Coefficient***	**Mean**
Carpus[a] (A3-A4/A5-A6)	174.092	1.929	175.831	2.011	0.52	174.72
Postion of hock w.r.t tuber ischiadicum (Horiz. P6 -> P9)	2.813	1.529	1.539	1.256	-0.52	2.45
Pastern / Metacarpus (A2 -> A3 / A3 -> A4)	0.549	0.088	0.595	0.071	0.35	0.54
Base of neck / Height at withers (T3/A8)	0.798	0.019	0.808	0.021	0.30	0.82
Tuber coxae -> Tuber sacrale (A11-A10)	6.392	2.221	5.384	2.517	-0.27	6.18
Tarsus[a] (P3-P4 / P5-P7)	177.691	2.138	178.541	2.278	0.24	178.10
Width of pelvis (P8-P8)	29.173	3.721	30.556	3.254	-0.24	29.23
Tarsus (P3-P4 / P6-P7)	129.709	3.357	129.322	3.029	0.08	129.18
Carpus (A3-A4/A5-A6)	183.481	5.612	183.262	4.129	-0.03	183.11

* Absolute canonical coefficients. The mid-point between the group centroid scores, which may be used as a cutting point to assign a previously unclassified horse to a class group, was 0.48; a horse with a value >0.48 would be classified as a high-class horse and a horse with a value <0.48 would be classified as a low-class horse.

[a] In frontal view

Table 8. Total canonical structure of the discriminating function separating low-class horses (scores: 6.5-7.0) and high-class horses (scores: 9.0-9.5) within ***tölt*** and mean and SD within the comparison groups. (Angles in lateral view). Lengths and heights in cm. and angles in degrees.

	Low-class group (N = 21)		High-class group (N = 21)			All horses (n=98)
Parameters	**Mean**	**SD**	**Mean**	**SD**	**Coefficient**[*]	**Mean**
Length of croup / Height at withers (P9 -> P10) / (P12)	0.364	0.015	0.347	0.014	0.64	0.35
Height at withers - height at croup (A8-A10)	3.980	2.404	5.611	2.132	-0.45	4.74
Height at withers (A8)	135.008	3.334	137.226	3.026	-0.44	136.23
Front fetlock joint (A2-A3-A4)	144.648	4.844	141.445	4.886	0.42	142.01
Height at croup - height at base of withers (A10-A9)	2.448	2.489	1.439	1.712	0.31	1.81
Base of neck / Height at croup (T3/A10)	0.824	0.028	0.835	0.016	-0.31	0.82
Length of back / Length of croup (A8 -> A10/P10 -> P9)	1.184	0.079	1.220	0.104	-0.26	1.21
Neck length / Height at withers (T2 -> T3) / (A8)	0.469	0.018	0.477	0.024	-0.25	0.48
Length of tibia (P5 -> P7)	41.928	2.272	42.576	2.479	-0.18	42.54
Length of back (A8 -> A10)	77.827	4.704	77.204	4.083	0.09	77.33
Inclination of scapula (A7-A8-Horiz.)	56.335	3.401	55.919	2.595	0.09	56.20

* Absolute canonical coefficients. The mid-point between the group centroid scores, which may be used as a cutting point to assign a previously unclassified horse to a class group, was 0.00; a horse with a value >0.00 would be classified as a low-class horse and a horse with a value <0.00 would be classified as a high-class horse.

Table 9. Total canonical structure of the discriminating function separating low-class horses (scores: 6.5-7.0) and high-class horses (scores: 9.0-9.5) within **slow *tölt*** and mean and SD within the comparison groups. (Angles in lateral view). Lengths and heights in cm. and angles in degrees.

	Low-class group (N = 30)		High-class group (N = 30)			All horses (n=98)
Parameters	**Mean**	**SD**	**Mean**	**SD**	**Coefficient***	**Mean**
Height at withers - height at croup (A8-A10)	4.102	2.360	5.692	2.109	0.60	4.74
Width of pelvis (P8-P8)	27.977	3.226	30.282	3.958	0.54	29.23
Height at croup - height at base of withers (A10-A9)	2.279	2.301	0.962	2.074	-0.51	1.81
Length of femur (P7 -> P8)	37.782	1.664	36.899	1.615	-0.46	37.53
Base of neck / Height at croup (T3/A10)	0.829	0.029	0.839	0.021	0.35	0.82
Hip joint (P7-P8/P9-P10)	86.496	4.294	87.803	3.668	0.28	86.53
Neck length / Height at withers (T2 -> T3) / (A8)	0.471	0.021	0.484	0.028	0.14	0.48
Tarsus (P3-P4/P6-P7)	129.861	3.742	130.212	2.776	0.09	129.18
Inclination of tibia (P7-P6-Horiz.)	44.616	2.717	44.773	2.687	0.05	44.07

* Absolute canonical coefficients. The mid-point between the group centroid scores, which may be used as a cutting point to assign a previously unclassified horse to a class group, was 0.00; a horse with a value >0.00 would be classified as a high-class horse and a horse with a value <0.00 would be classified as a low-class horse.

Table 10. Total canonical structure of the discriminating function separating horses with scores of 5.0 and high scores (8.0-9.5) for **pace** and mean and SD within the comparison groups. (Angles in lateral view). Lengths and heights in cm. and angles in degrees.

	Low-class group (N = 20)		High-class group (N = 28)			All horses (n=98)
Parameters	**Mean**	**SD**	**Mean**	**SD**	**Coefficient***	**Mean**
Length of croup / Height at withers (P9 -> P10) / (P12)	0.346	0.018	0.361	0.012	-0.60	0.35
Height at base of withers / height at withers (A9/A8)	0.948	0.009	0.964	0.009	-0.42	0.95
Pastern / Metacarpus (A2 -> A3 / A3 -> A4)	0.572	0.073	0.523	0.088	0.39	0.54
Metacarpus / Height at withers (A3 -> A4 / A8)	0.139	0.008	0.144	0.007	-0.34	0.14
Postion of hock w.r.t tuber ischiadicum (horiz. P6 -> P9)	1.838	1.653	2.583	1.571	-0.31	2.45
Length of back / Length of croup (A8 -> A10/P10 -> P9)	1.229	0.116	1.193	0.061	0.28	1.21
Height at croup - height at base of withers (A10-A9)	2.104	2.166	1.471	1.810	0.21	1.81
Inclination of pelvis (P9-P10-Horiz.)	22.034	1.883	22.719	2.783	-0.19	21.82
Length of neck / Height at withers (T2 -> T3 / A8)	0.564	0.035	0.556	0.044	-0.05	0.56
Inclination of tibia (P7-P6-Horiz.)	44.244	3.533	44.492	2.785	-0.05	44.07
Base of neck / Height at withers (T3/A8)	0.805	0.018	0.813	0.014	-0.03	0.82

* Absolute canonical coefficients. The mid-point between the group centroid scores, which may be used as a cutting point to assign a previously unclassified horse to a class group, was 0.09; a horse with a value >0.09 would be classified as a low-class horse and a horse with a value <0.09 would be classified as a high-class horse.

Table 11. Total canonical structure of the discriminating function separating low-class horses (scores: 6.5-7.0) and high-class horses (scores: 8.5-9.5) for **pace** and mean and SD within the comparison groups. (Angles in lateral view). Lengths and heights in cm. and angles in degrees.

	Low-class group (N = 20)		High-class group (N = 20)			All horses (n=98)
Parameters	**Mean**	**SD**	**Mean**	**SD**	**Coefficient**	**Mean**
Hip joint (P7-P8/P9-P10)	84.663	3.669	89.755	4.590	0.61	86.53
Femur to the horizontal plane (P7-P8-Horiz.)	62.164	3.519	64.989	4.304	0.40	62.96
Inclination of pelvis (P9-P10-Horiz.)	21.182	2.777	23.007	2.724	0.37	21.82
Length of croup / Height at withers (P9 -> P10) / (P12)	0.353	0.014	0.363	0.013	0.36	0.35
Length of radius (A5 -> A6)	28.959	1.206	29.665	1.146	0.34	29.01
Stifle joint (P5-P7-P8)	119.15	4.55	122.25	7.16	0.30	119.73
Height at croup - height at base of withers (A10-A9)	2.389	2.779	1.208	1.623	-0.29	1.81
Base of neck / Height at withers (T3/A8)	0.798	0.022	0.814	0.016	0.25	0.82
Length of tibia (P5 -> P7)	42.737	1.941	42.082	1.822	-0.20	42.54
Length of back / Length of croup (A8 -> A10/P10 -> P9)	1.199	0.076	1.179	0.064	-0.16	1.21
Humerus / Height at withers (A6 -> A7) / (A8)	0.199	0.013	0.202	0.014	0.11	0.20
Length of back (Horiz. 11 -> 12)	64.587	3.259	65.204	2.984	0.11	64.85
Pastern / Metacarpus (A2 -> A3 / A3 -> A4)	0.543	0.104	0.550	0.097	0.05	0.54
Radius / Metacarpus (A5->A6 / A3->A4)	1.524	0.114	1.530	0.119	0.03	1.53

* Absolute canonical coefficients. The mid-point between the group centroid scores, which may be used as a cutting point to assign a previously unclassified horse to a class group, was 0.05; a horse with a value >0.05 would be classified as a high-class horse and a horse with a value <0.05 would be classified as a low-class horse.

Supplementary table. Attritbutes assessed in the scoring of gaits in breeding field tests for Icelandic horses and their weight in the total score.

Trait	Advantages	Disadvantages	Weight, %
Walk	Four-beat, suppleness, length of strides, energy	Trotty or pacey beat, stiffness, short stride length, lack of energy	4.0
Trot	Two-beat, suspension, length of strides, speed capacity, high leg action	Four-beat, lack of suspension, lack of speed, short stride length, low action	7,5
Gallop	Four-beat, stretch, suspension, speed capacity	Lack of speed, lack of suspension, heavy movements	4,5
Canter	Three-beat, suspension, length of stride, suppleness	Four-beat, lack of suspension, short stride length, low leg action	-
Tölt	Four-beat, suppleness, length of strides, speed capacity, high leg action	Trotty or pacey beat, stiffness, short stride length, lack of speed, low leg action	15.0
Slow tölt	Four-beat, suppleness, length of strides, high leg action	Trotty or pacey beat, stiffness, short stride length, low leg action	-
Pace	Close to two-beat, suspension, speed capacity, stride length	Four-beat, lack of suspension, lack of speed, short stride length	10.0

The conformation and the riding ability traits have a 40% and 60% weight in the total score, respectively. The traits of riding ability not listed in the table are spirit (weight: 9%) and general impression (weight: 10%).

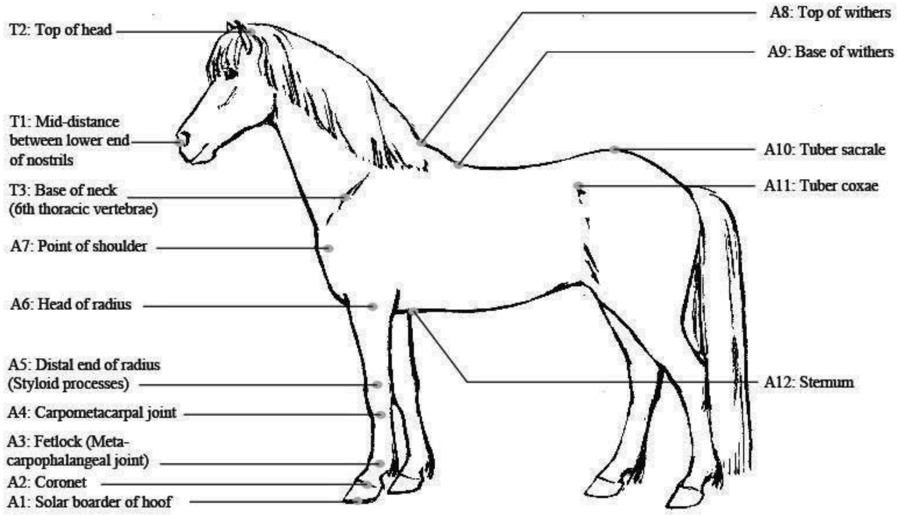

Figure 1.

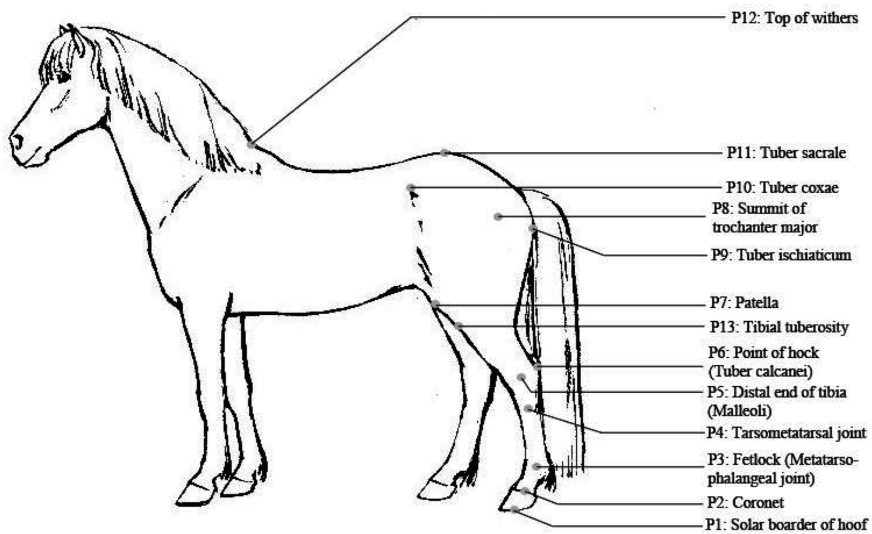

Figure 2.

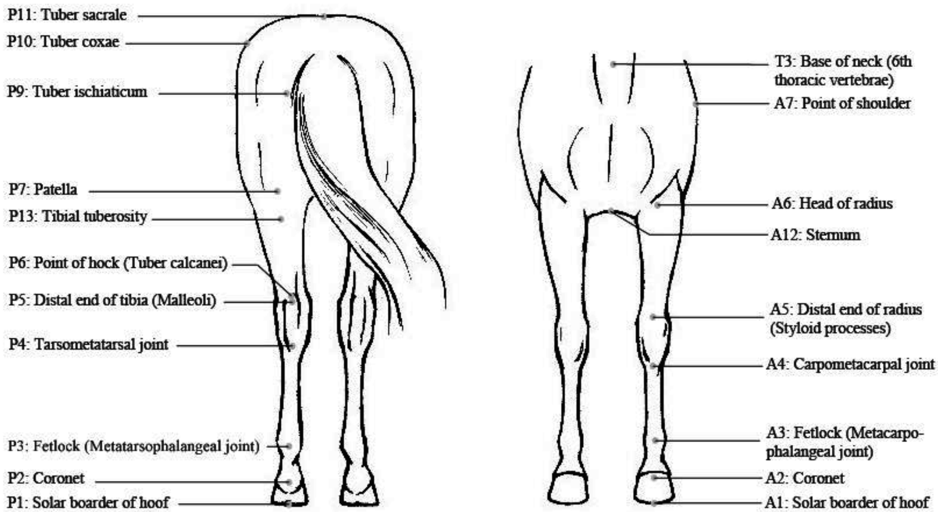

Figure 3.

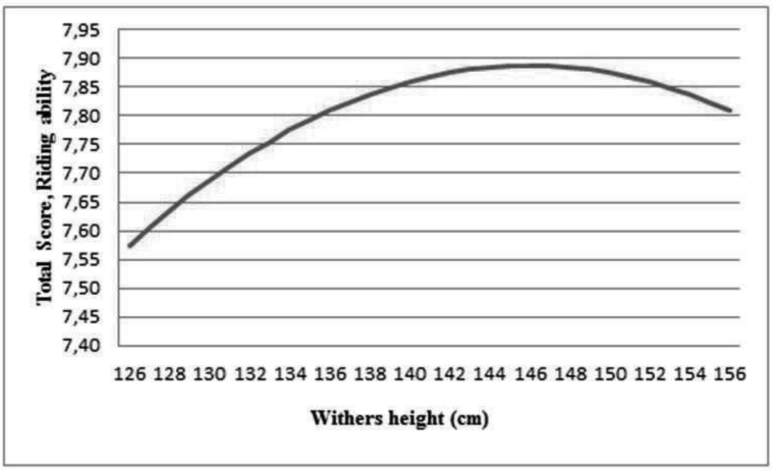

Figure 4.