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GENETIC OF LINEAR TYPE TRAITS AND LIFETIME FERTILITY RATE IN ITALIAN HEAVY DRAUGHT HORSE

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RIASSUNTO GENERALE

Il Cavallo Agricolo Italiano da Tiro Pesante Rapido (CAITPR) è comunemente selezionato sulla base dei caratteri lineari valutati in giovane età sui nuovi puledri nati, quando hanno un'età approssimativa di 6 mesi. Questo schema rappresenta una sorta di performance test a livello di popolazione, che permette la valutazione genetica di stalloni e fattrici per i caratteri legati al principale obiettivo di selezione (tiro pesante e produzione di carne) realizzando una valutazione fenotipica in vita in età precoce. Gli animali trattenuti per la riproduzione sono comunque punteggiati anche a 30 mesi circa di età, per l'ammissione ufficiale al libro genealogico. I dati rilevati sui caratteri lineari ottenuti dagli adulti non sono mai stati sottoposti ad analisi per il fine della valutazione genetica. Come scopo generale, questa tesi si è proposta di realizzare i seguenti obiettivi: condurre le analisi genetiche dei caratteri lineari ottenuti dai puledri di CAITPR, analizzare i caratteri lineari valutati sugli adulti, fattrici e stalloni, effettuare una comparazione genetica degli stessi caratteri rilevati a differenti età. Inoltre, a causa del crescente interesse per i caratteri legati alla fertilità nei programmi di selezione, questo studio ha avuto lo scopo di implementare un tasso di fertilità variabile, usato per le analisi della sua componente genetica, ai fini di una sua possibile implementazione nel presente schema di selezione del CAITPR.

Per il primo studio è stato estratto dalla banca dati dell'associazione nazionale del CAITPR un ammontare di 17,725 dati di caratteri lineari registrati sui puledri a partire dal 1997. A causa dell'ammontare di record con meno di due puledri figli di diverso stallone per singolo livello di azienda-anno-valutatore (AAV), sono stati confrontati 6 differenti metodi di raggruppamento delle aziende ai fini di determinare quale si adattasse meglio al modello. Inoltre, questo studio ha avuto lo scopo di stimare l'ereditabilità e le correlazioni genetiche tra i caratteri lineari. Il dataset finale usato consisteva in 11,357 punteggi di giovani puledri (7,350 femmine e 3,287 fattrici), riferiti a 17,441 soggetti nel pedigree. Sulla base del *Likelihood ratio test*, tutte e 6 le differenti combinazioni di aziende nelle celle ambientali hanno mostrato una significativa influenza nell'adattamento del modello, ma le statistiche basate sull'analisi dei residui (*percentage square biases, mean absolute deviation, residual standard deviation*) hanno indicato un

adattamento migliore per la combinazione con maggiore complessità, indipendentemente dai maggiori gradi di libertà. Le analisi genetiche *single-trait*, effettuate considerando alternativamente la l'effetto ambientale fisso o random, hanno suggerito un maggior adattamento per il modello con l'effetto ambientale fisso, maggiormente adatto quindi alle analisi. Le analisi genetiche hanno rilevato che il carattere più ereditabile è stato la muscolosità (0.355), mentre il carattere che ha esibito la più bassa ereditabilità è stato la direzione della linea dorsale (0.048). I 5 caratteri combinati nel presente indice di selezione, (distinzione, temperamento, muscolosità, diametri anteriori e posteriori) hanno mostrato i maggiori valori di ereditabilità, variando dal 0.355 al 0.198. Anche lo sviluppo generale ha mostrato un buon livello di ereditabilità, sebbene vada considerato come un carattere composito, che include statura, diametri anteriori e posteriori, profondità toracica e altezza al ginocchio. Le correlazioni genetiche trovate in questo studio sono risultate elevate tra muscolosità e diametri posteriori (0.929), e leggermente inferiori tra muscolosità e diametri anteriori (0.833). La più bassa correlazione stimata è stata tra distinzione e incidenza dell'impalcatura ossea. (-0.600).

Il secondo studio è stato effettuato con l'obiettivo di analizzare per la prima volta la componente genetica dei caratteri lineari punteggiati in età adulta, analizzando anche il *trend* genetico indiretto ottenuto come effetto della selezione effettuata dagli anni 90', sulla base degli EBV stimati nei puledri. Un ammontare di 7,133 dati riguardanti i caratteri lineari valutati annualmente sugli esemplari adulti è stato ottenuto dalla banca dati del libro genealogico. Il dataset preparato per le analisi includeva i singoli rilievi di 6,691 soggetti (5,835 femmine e 856 maschi) con un'età di 30 mesi circa. Gli animali sono punteggiati linearmente per 14 caratteri con una scala a 9 punti (da 1 insufficiente, a 5 eccellente, inclusi i mezzi punti), da 33 valutatori in 21 anni consecutivi di valutazioni (dal 1992 al 2013); il punteggio finale espresso dai valutatori è stato anch'esso considerato nelle analisi, diventando il 15° carattere nella valutazione genetica. L'analisi genetica ha riguardato un ammontare di 11,012 animali nel pedigree. L'effetto dato dalla combinazione dell'azienda o del gruppo di aziende con l'anno e il valutatore è stato considerato quello che nello studio precedente ha dato il miglior adattamento. L'ereditabilità stimata per i caratteri lineari rilevati negli adulti variava da 0.03 (lunghezza linea dorsale) a 0.40 (sviluppo generale). Le ereditabilità dei due caratteri legati all'aspetto esteriore, distinzione e temperamento, sono risultate di magnitudine medio-bassa, rispettivamente 0.32 e 0.21. Simile stime sono state ottenute per i caratteri relativi alla muscolosità, cioè la muscolosità stessa, e i diametri anteriori e posteriori, i cui valori di ereditabilità variavano dal 0.25 al 0.32. Tutti i caratteri relativi alla conformazione e correttezza hanno mostrato bassi valori di ereditabilità (dal 0.04 al 0.13), mentre il punteggio finale ha raggiunto un'ereditabilità di 0.30. Sono state ottenute correlazioni genetiche (r_g) elevate tra i caratteri relativi allo sviluppo muscolare: muscolosità e diametri posteriori (r_g=0.91), muscolosità e diametri anteriori (r_g=0.74), diametri anteriori e posteriori (r_g=0.76). Un incremento del valore genetico nel tempo è stato riscontrato nei caratteri interessati dalla selezione (muscolosità, distinzione e temperamento), nonostante non sia mai stata effettuata una selezione diretta basata sui caratteri lineari a 30 mesi. Lo studio ha mostrato una significativa variabilità genetica in molti dei caratteri analizzati, suggerendo che i caratteri lineari punteggiati negli animali adulti invece che nei puledri potrebbero essere efficacemente utilizzati nei piani di selezione del CAITPR.

In una terza fase, la tesi si è focalizzata sull'analisi delle correlazioni genetiche tra i caratteri punteggiati nei puledri e negli adulti, considerando anche gli effetti prodotti dalla selezione in seguito all'uso dei caratteri lineari alternativamente ottenuti dai puledri o dagli adulti. Il dataset iniziale consisteva in 7,133 valutazioni di adulti e 15,495 di puledri punteggiati da 33 valutatori in 21 anni consecutivi (dal 1992 al 2013), su 11 dei 14 caratteri lineari con una scala di 9 punti (da 1 a 5 includendo i mezzi punti). A seguito dell'editing dei dati sono stati considerati per le successive analisi 6,691 punteggi negli adulti e 11,357 punteggi nei puledri, quindi uniti in un unico dataset comprendente 13938 animali di cui 4110 valutati in entrambe le età. Le analisi, effettuate su modelli bivariati applicando un algoritmo di *gibbs-sampling,* sono state realizzate su un totale di 18,048 dati relativi a 18,773 animali nel pedigree. Il modello considerava a i seguenti effetti fissi: allevamento (gruppo)-anno di valutazione-valutatore (2,637 classi per i puledri e 1,297 per gli adulti), sesso (2 classi), età alla valutazione (9 classi per i puledri e 5 per gli adulti), e età della madre al parto (5 classi solo per i dati dei puledri). Le correlazioni genetiche tra i caratteri misurati nei puledri e negli adulti variavano dal -0.67 al 0.92, e quelle fenotipiche -0.19 al 0.47. le più basse correlazioni tra adulti e puledri sono state osservate sui caratteri di distinzione e incidenza dell’impalcatura ossea (-0.67). Le correlazioni stimate per gli stessi caratteri valutati sia nei puledri che negli adulti sono risultate particolarmente elevate per i caratteri di sviluppo generale (0.92), direzione della linea dorsale (0.88) distinzione (0.87) e incidenza dell’impalcatura ossea (0.86). Le correlazioni tra i caratteri legati alla muscolosità (muscolosità diametri anteriori e posteriori) variavano invece da 0.55 a 0.66. Nello studio sono state inoltre confrontate tra loro delle stime di progresso genetico ottenute tenendo conto della selezione diretta per i 5 caratteri considerati nell’indice di selezione complessivo (distinzione, temperamento, muscolosità, diametri anteriori e posteriori) e alternativamente basate sugli EBVs dei puledri e degli adulti. I risultati hanno indicato un trend atteso di generazione simile nei due scenari (selezione basata sugli EBVs dei puledri e adulti). A causa però del basso intervallo generazionale permesso grazie all’uso dei punteggi registrati in giovane età, l’utilizzo dei puledri in ambito selettivo potrebbe portare ad un trend maggiore rispetto a quello che conta solo sul progresso genetico ottenibile usando solo i caratteri valutati negli adulti. Quindi, la scelta di usare i caratteri lineari degli adulti anziché quelli dei puledri, sebbene fattibile, non può essere considerata una soluzione efficiente.

Lo scopo del quarto studio è stato trovare una variabile fenotipica capace di esprimere le prestazioni riproduttive in vita delle fattrici a un dato punto finale, e di analizzare la componente genetica del carattere in modo da considerare la possibile implementazione della valutazione della fertilità nell’ambito della gestione della razza CAITPR. In un primo step dello studio sono state considerate 1,487 fattrici nate dopo il 1990. Le fattrici sono state scelte seguendo i seguenti criteri: l’aver avuto almeno 6 eventi riproduttivi consecutivi registrati (RS), l’appartenere ad una cella ambientale con almeno due osservazioni (gruppo o allevamento nella stessa area geografica, e sistema di allevamento comune e stesso anno di nascita), e avere i genitori noti. Questo *training dataset* è stato usato per implementare una serie di coefficienti o equazioni per stimare la produzione di puledri (FP) al 6° RS in relazione al precedente FP (dopo 3,4 o 5 RS), e tenendo conto dell’età al primo parto (3 o 4 anni quando considerati). La validazione dei coefficienti, o equazioni predittive, è stata effettuata analizzando gli scarti medi ottenuti usando le differenze individuali tra il tasso di puledri prodotti in vita attuale e stimato (LFR, cioè l’FP attuale o stimato diviso per le 6 opportunità di parto). Un ulteriore dataset, comprendente 3,033 fattrici scelte sulla base dei precedenti criteri, ma con almeno 3 eventi RS registrati, è stato usato per predire la produzione di puledri al 6° RS per le fattrici con 3,4 o 5 RS registrati. Questo dataset conteneva i tassi di produzione di puledri attuale (n=1,950) e stimato (n=1,443), ottenuti entrambi con coefficienti (LFR-C) e con equazioni (LFR-E)..E' stata inoltre considerata la trasformazione arcoseno del LFR. L'ereditabilità dei caratteri è stata ottenuta mediante *single-trait animal model* e utilizzando un pedigree di 6,803 animali. Gli EBVs ottenuti sono stati quindi usati per confrontare i 4 differenti LFR in termini di andamento genetico e correlazioni tra i ranghi. Le equazioni di previsione risultavano ben validate quando le equazioni indipendenti di regressione lineare tenevano conto dell'età al primo parto per stimare il LFR al 6° RS, e presentavano bassi valori, indice di validità del modello, di *percentage square bias* (0.0094), *mean absolute deviation* (0.0583) e *residual standard deviation* (0.0769). Il tasso stimato di puledri prodotti ha mostrato una varianza genetica moderata ma significativa, e l'ereditabilità del carattere LFR è risultata medio bassa e pari a 0.25. La trasformazione in arcoseno di entrambi LFR-C e LFR-E non ha mostrato incrementi di ereditabilità, sebbene l'adattamento del modello incrementi leggermente (valori più bassi du -2 Log likelihood). Le correlazioni dei ranghi degli EBVs per fattrici con dati fenotipici o per stalloni con ≥9 figli sono risultate estremamente elevata ponendo a confronto le 4 differenti LFR (la media del coefficiente di correlazione è di 0.996), e, come atteso, il trend genetico risulta invariato per le 4 differenti espressioni del LFR, sebbene costante per stalloni e fattrici. Lo studio ha indicato il possibile uso per scopi selettivi nel CITPR della variabile lineare LFR espressa come valore attuale o stimato alla 6° stagione riproduttiva per carriere incomplete. Comunque, successive analisi volte a valutare la relazione tra la fertilità e gli altri caratteri produttivi, saranno necessarie prima dell'implementazione pratica di un miglioramento genetico per la fertilità nel CAITPR.

GENERAL ABSTRACT

The Heavy Draught Horse (IHDH) breed is commonly selected on the basis of linear type traits evaluated yearly at young age on the new born foals when aged approximately 6 months. This scheme represent a sort of performance test at population level that allows the genetic evaluation of stallion and mares for traits related to the main selection goals (i.e., heavy draught and meat production) from phenotypes of their products evaluated early in life. Animals retained for reproductions are however scored also at about 30 months of age, i.e., at the official admission to the stud book, but data on type traits obtained on adults have never been analysed. The general aims of this thesis have been the genetic analysis of linear type traits obtained on young foals, the type traits evaluated on adults IHDH mares and stallions and the genetic comparison of the same traits scored at different ages. Additionally, due to the increasing interest for fertility traits to be used for breeding purposes, this study has aimed also at implementing a lifetime fertility rate variable to be used for the analysis of its genetic components aimed at analysing its possible implementation in the present selection scheme of the IHDH breed.

For the first study an amount of 17,725 records on type traits recorded on foals since 1997 were preliminarily extracted from the database of the national breeders association of IHDH. Because of the small number of records within single levels of stud-farms x year x classifier (SYC; average of 2 records), 6 different method of grouping the stud-farms were applied and analysed for their contribution to the model fitting. Subsequently, this study has aimed at evaluating the heritability and genetic correlation among type traits. The final dataset use consisted in 11,357 scores of young foals (7,530 females and 3,827 males), and accounting for 17,441 subjects in the pedigree files. On the basis of a likelihood ratio test, all the 6 different combinations of stud-farms in environmental groups showed a significant influence on the model fitting, but the percentage square biases, the mean absolute deviation and the standard deviation of residues, indicated in the combination with the greater complexity, a general better fitting, independently form the increased degrees of freedom. The genetic analysis carried out on single trait considering alternatively the SYC

effect as fixed or random; indicate that the first approach was better suitable than the latter one, because of the increased fitting. The genetic analysis revealed that the most heritable trait was fleshiness (0.355), while the trait exhibiting the lower heritability was the upper line direction (0.048). However the 5 traits combined in the present selection index, (head size, temperament, fleshiness, fore and rear diameters) showed the greater heritability values, ranging from 0.355 to 0.198. Also the frame size showed a good heritability value (0.257), although it has to be considered a composite trait, accounting for stature, fore and rear diameters, thorax depth and knee height. The genetic correlations founded in this study, indicate a great correlations among traits linked to fleshiness. The greatest value was found indeed between fleshiness and rear diameters (0.929), while the correlations among fleshiness and the fore diameters was a little lower (0.833). The lower correlation estimated was that between head size e bone incidence (-0.600).

The second study was carried out with the objective of analysing or the first time the genetic components of type traits scored at the adult age, analysing also the indirect genetic trends obtained as effect of the selection carried out since the 90’s on the basis of EBVs estimated on young foals. An amount of 7,133 records were obtained from stud book database of IHDH breed and relative to the linear type traits evaluation annually recorder. The dataset suitable for analyses included single records of 6,691 subjects (5,835 females, 856 males), aged about 30 months. Animal were scored linearly for 14 traits with a 9 point scale system (from 1 unfair, to 5 excellent, including half points) by 33 classifiers in 21 subsequent years of evaluation (i.e. from 1992 to 2013); the final score expressed by classifier was also considered in this analysis, becoming the 15th trait under genetic evaluation. The genetic analysis accounted for 11,012 animals in pedigree file. The combination of stud or group of stud combined by year and classifier was considered the one that gave the best fitting in the previous study, The heritability estimates obtained for adult type traits ranged from 0.03 (upper line length) to 0.40 (frame size). The heritability of the two traits related to outward appearance, i.e., head size expression and temperament resulted of medium-low magnitude, i.e., 0.32 and 0.21, respectively. Similar estimates were obtained for traits related to fleshiness, i.e., the fleshiness itself, the fore and rear diameters, whose h^2

values ranged from 0.25 to 0.32. All traits related to conformation correctness showed low heritability values (i.e., from 0.04 to 0.13), while the final score reached a heritability of 0.30. High genetic correlations (r_g) were obtained among traits related to muscular development: fleshiness and rear diameters (r_g=0.91), fleshiness and fore diameters (r_g=0.74), and fore and rear diameters (r_g=0.76). Positive genetic trends were found in traits of selection interest (fleshiness traits, head size and temperament) in spite of the fact that a direct selection based on 30 months type evaluation has never been carried out. The study showed a significant genetic variance for many analysed traits, suggesting that type traits scored in adult animals instead in foals could be a feasible method for the IHDH selection plan.

In the third step, the thesis was focussed on the analysis of genetic correlations between traits scored both at young an adult age, aiming also at analysing the effect produced by selection considering the alternative use of type traits obtained on young or adult animals. The initial databases consisted in 7,133 evaluations of adults and 15,945 of foals scored by 33 classifiers in 21 subsequent years (i.e. from 1992 to 2013) on 14 linear type traits with a 9 point scale system (from 1 to 5 including half points). After editing, 6,691 scores on adults and 11,357 scores on foals were retained for further analysis. Data were merged and 4,110 animals resulted scored at both ages. Bi-traits Gibbs Sampling analyses were carried out on a total of 18,048 records related to 18,773 animals in the pedigree. The model considered the following fixed effects: stud (group)-year-classifier (2,637 classes for foals and 1,663 for adults), sex (2 classes), age at evaluations (9 classes for foals and 5 for adults), and age of mare at foaling (5 classes, for foal data only). The genetic correlations between traits measured in young and adult age ranged from -0.67 to 0.92, and the phenotypic correlations ranged from -0.19 to 0.47. The lowest genetic correlations between foal and adult scores were observed on head size and bone incidence (i.e., -0.67). Greater genetic correlations were estimated for the same traits evaluated at young and adult age particularly on frame size (0.92), upper line direction (0.88), head size (0.87), bone incidence (0.86). While the correlation among traits correlated with muscularity (fleshiness, fore and rear diameters) ranged from 0.55 to 0.67. The correlation among overall score and traits score in the foals, showed medium low values, ranging from

0.02 to 0.66. An estimate of the genetic progress obtainable accounting for direct selection for the 5 traits at present considered in the total merit index (head size and expression, temperament, fleshiness, fore and rear diameters), and based on the use of EBVs from young foals or adults were compared. Results indicate a similar expected genetic trend for generation both using EBVs from young or adult horses. However, due to the lower generation interval allowed by the use of scores on young foals, a greater yearly genetic trend could be expected in this scenario as compare to the one accounting only on the genetic progress attainable using type traits scored on adults. Therefore, the choice of using the type traits instead of those obtained on foals, although feasible, cannot be considered an efficient solution.

The aims of the fourth study were to find out a phenotypic variable able to express mares' lifetime reproductive performance at a given endpoint and to analyze the genetic components the trait in order to exploit the possible implementation of a breeding for fertility process in the IHDH breed. In a first step of this study 1,487 mares born after 1990 were considered. Mares had at least 6 subsequent registered reproductive seasons (RS), and belonged to environmental units with at least 2 observations (group of studs in the same geographical area and common rearing system by year of birth), and had known parents. This training dataset was used to implement a set of predictive coefficients or equations to estimate foal production (FP) at the 6^{th} RS depending on previous FP (i.e., after 3, 4 or 5 RS), and accounting or not for the age at first foaling (3 or 4 years when considered). The validation of predictive coefficients or equations was carried out by analyzing the mean biases using individual differences between actual and estimated lifetime foaling rate (LFR, i.e., the actual or estimated FP divided by the 6 opportunities of foaling). A further dataset accounting for 3,033 mares and obtained following the same editing restriction as above, but with at least 3 registered RS, was used to estimate the foaling rate at the 6th RS for mares with 3, 4, or 5 registered RS. This dataset contained actual (n=1,950) and estimated (n=1,443) LFR, obtained both with coefficients (LFR-C) and with equations (LFR-E). Arcsine transformed LFR were also analyzed. Heritability in single trait animal model accounting for 6,803 animals in the pedigree file was estimated and EBVs obtained were used to compare the 4 different LFR in term of genetic trends and rank correlations.

The predictive equations resulted well validated when separate linear regression equations accounting for the age at first foaling were used to estimate LFR at 6th RS, with lower percentage square biases (0.0094), mean absolute deviation (0.0583) and standard deviation of residuals (0.0769). The estimated foaling rate showed a moderate but significant genetic variation, and the heritability of the trait resulted medium-low, i.e., 0.25. The arcsine transformation of bot LFR-C or LFR-E did not show any improvement of heritability, although increased slightly the fitting of the model (i.e., lower -2 Log likelihood). Rank correlations of EBVs for mares with phenotypic records or for sire with ≥9 daughters resulted extremely high comparing the 4 different LFR (average correlation coefficient of 0.996), and, as expected, genetic trends resulted unchanged for the 4 different LFR expression, although almost steady for both mares and stallions. The study indicates the possible use for breeding purposes in IHDH horse of a linear LFR variable expressed as actual or as estimated value at the 6th reproductive season for incomplete career. However, further analyses aimed at evaluating the relationship of fertility with the other productive traits are needed before the practical implementation of a breeding process for fertility in IHDH.

CHAPTER 1

GENERAL INTRODUCTION

1.1 HISTORY

An increasing body of archaeological evidences on the beginning of horse domestication locates the origin of *Equus Ferus Caballus* in the steppes of in eastern Eurasia, which is in the modern-day the Ukraine and Kazakhstan territory. Here, some of the earliest archaeological evidences for managed horse population have been found, providing additional evidence to the worth of this region as a primary area for horse domestication (Warmuth et al., 2012).

Horses have been initially reared for military and agricultural purposes, but during the centuries the goals of selection have been changed, and nowadays horses are reared mainly for sport, draught and meat production.

The Italian Heavy Draught Horse (IHDH) was initially developed within military programme that can be traced back to the beginning of the Italian country (i.e., 1861), aiming at obtaining a heavy strain of horse to be used both for rapid draught in agriculture and field artillery. Over the years the goal selection changed until present day, now the mean goal of selection is the meat production, while the secondary selection goal is the heavy draught (Mantovani et al., 2005)

1.2 GENETIC IMPROVEMENT IN HORSE BREEDS

The problems related to the genetic improvement of horse breeds as respect to other livestock species are various and dependent mainly on the breeding characteristics of this specie, that is distinguished by a low rate of reproduction, long generation intervals, and the presence of overlapping age groups among breeding candidates.

In spite of this, most horse breeds today have in specific breeding plans aimed at increasing aspects related with the economic layout of their activity. In other words, the identification of quantitative traits of economic interest to be improved through a breeding scheme is the main target of selection in the horse industry. In some instances, more than a trait is recognized of economic interest for selection, and is this case, economic weights are applied to each trait of interest in order to obtain a single index to be used for animal selection.

At present, researches are needed for determining the economic values many traits to be used in the genetic improvement of horse populations, and more generally for determining the relative weight among different breeding objectives in a given breeding scheme.

The correct general direction of the breeding goal in a population is of course of greatest importance, while slight deviations of economic weights from the correct ones have a limited impact on selection efficiency.

For example, the official breeding goal of the Iceland horse consist of a linear function of seven conformation traits and seven riding performance traits which are scored in special field performance test. The production of horses to be used as draught animals in agriculture and forestry may require breeding goals including pulling power, pulling technique and temperament. On the other hand, the breeding goal for hackney horses should include movement, temperament and elegance. The general rule is that the breeding goal should include those traits which are of real functional importance for the breed, and should avoid wasting selection pressure on redundant traits.

Most traits included in breeding objectives for horses are influenced by many genes and many environmental factors. The purpose of genetic evaluations is to estimate the breeding value of each individual animal. The breeding value is defined as the sum of average effects of the gene affecting a particular quantitative trait (Ricard et al., 2000)

The use of selection index (BLUP) principles for genetic evaluation of horses started in the mid-1970s with the study if Langlois (1975) for French riding horses and with the research carried out by Minkema (1976) in for racing trotters in the Netherlands.

The rate of genetic improvement in a population under selection can be predicted if the following four factors are known: i) genetic variance for the trait under selection; ii) its heritability; iii) the intensity of selection for this criterion; and iv) the generation interval in both males and females Falconer (1989).

Selection is therefore expected to be effective for changing the population mean in the desired direction for many generations, provided that deleterious effects on inbreeding are avoided and that the effective population size is kept large enough. Sensible animal breeding is a fantastic process for sustainable utilization of biological resources.

The effect of selection on changing the genetic variance over generation was considered previously. The conclusion was that variance in quantitative traits amongst the annual crops of new-born foals is reduced somewhat over time with increased intensity of selection until equilibrium is reached and that thereafter genetic variance remains unchanged.

Many horse populations are small and animal breeding principles that are optimal in large populations may not be applied correctly.

In small populations, for example, the gene frequencies may fluctuate randomly from generation to generation as a result of the finite sampling of gametes. This phenomenon, called genetic drift, is quantified by the term effective population size N_e.

It has been hypothesized that in the future, the possible use of marker-assisted selection (MAS) could enhanced genetic improvement in horses. Interest in MAS is likely to be a valuable complement to selection on EBVs obtained by the BLUP method, rather than as a replacement for EBVs. The use of MAS in horse breeding schemes could be particularly useful for traits that are expressed late in the horse’s life because the genetic markers will become known in the foal. The effect of MAS would be to shorten the generation interval and thereby increase the rate of genetic progress. However, at present, evidence for the existence of quantitative traits loci (QTL) with large effect (major genes) in horse is scarce.

Recently, health traits and longevity have received increasing attention as a part of the breeding goal for many horse breeds.

1.3 LINEAR TYPE EVALUATION

Conformation of horses can be defined as its form or outline. The conformation of today's horse is a result of both nature's selection and selection carried out by man for various purpose; it varies among different breeds and even between different subpopulation and lines belonging to the same breed.

A quantitative approach to conformation traits was undertaken in the 18th century by Bourgelat (1754) who studied linear measurements of the body segment of the baroque horse. In the 19th and early 20th century, other scientific studies developed hippometric methods and took into account the joint angles and inclination of the limb segments as interest traits (Saastamoinen et al., 2000)

Nowadays the description of a horse's conformation and its details are presented in many handbooks.

Therefore, judging conformation has long-standing traditions in horse breeding. Although selection of horses is based mainly on their performance results, conformation and gaits still have an important role in judging horses at stud book shows. However, today's horse is mainly an athlete or working animal, and its value is determined largely by its exercise ability and the health of its limbs. Furthermore, correct conformation and good movements are important factors to obtain high prices for horses of all categories at auction.

A linear assessment of conformation trait has been proposed and implemented to allow quantitative description of the static conformation of the horse. A restricted number of objective conformation studies to estimate heritability have been carried out. However, the horse may have good conformation from one view and poor conformation from another. Criteria for describing the basic and ideal conformation of the horse are presented in many publications.

Linear type traits describe biological extremes for a range of visual characteristics of an animal and one of the primary reason for collecting them is to allow breeders to select the most functional and profitable animals (Mazza et al., 2014).

The linear type evaluation is a descriptive method that permits to describe the phenotypic characteristics of animals, by assigning scores that indicate the expression of trait. The linear type evaluations allows also the recruitment of large datasets to be used for the genetic analysis and the setting of breeding value estimates to be used in the selection process. This method was developed initially for cattle breeding (White, 1974; Thompson et al., 1981), and then it was applied to other livestock species. In the horses, many breeds introduced linear scoring system in the studbook, as Trakehner (Preisinger et al., 1991), the KWPN (Koenen et al., 1995), the Old Kladrub horse (Jakubec et al., 2007), the Belgian warmblood horse (Rustin et al., 2009), the Hanoverian warmblood (Schröde et al., 2010), the Andalusian (Molina et al., 1999; Sánchez et al., 2013), and many other European saddle horses from Germany, Denmark, Finland, Hungary, Ireland, The Netherland, Norway, France, Great Britain, Italy, and Sweden (see for review Koenen et al., 2004).

The draught horses mainly conformation traits are used for the genetic evaluation of animals. For example, Druml et al. (2007), used both body measurements and conformation traits, applying a linear scale from 1 to 10, in the Noriker draught horse. Fioretti et al (2005) applied a linear type classification system in the genetic evaluation of the Bardigiano horse breed, while Simčič et al. (2012), used measured and scored traits (1 to 10) for the Posavje horse. Instead of linear types, Zechner et al. (2001) used body measurements for Lipizzan horse. Bakhtiari et al. (2009) reported the use of conformation measurements in the Iranian thoroughbred horse, while Bhatnagar et al. (2011) used type traits with a scale from 1 to 10 for their study on the genetic parameters in the American sport horse. Also Jönsson et al., (2010), used linear score for conformation and gait scores, using a scale of from 1 to 10 for the Danish horse.

The IHDH selection goal is mainly focussed on the simultaneous improvement of both meat and heavy draught, although the main selection goal remains the meat production. However, in the recent years there has been an increasing interest for the rapid draught in the horse team competition and in the use of horses in agricultural works.

The introduction of linear type evaluation in the IHDH started in the 1992; subsequently, in the 2002, the committee for breeding program (i.e., Commissione tecnica centrale, CTC) reduce the traits evaluated in the foals from 14 to 11. In the 2004, also the half points in the scale of evaluation were introduced. In table 1 gives a deep description of the 14 type traits recorded on both foals (only the first group of 11) and adults.

The traits evaluated on the IHDH belong to three main groups: 1) general aspect (head size, temperament, frame size, muscularity, bone incident); 2) trunk (thorax depth, fore diameters, rear diameters, upper line length, upper line direction); 3) legs (legs side view, fore feet, rear feet, hind legs back view).

The animals are evaluated two times during their life, i.e., at 6 months of age (young foals), and at the time of official admission to the studbook, i.e., at about 30 months of age. While on young foals, 11 traits are linearly scored (excluded fore feet, rear feet and hind legs back view), adult are still scored for all the original 14 traits developed in 1992.

After the estimates of individual breeding values, a selection index is calculated for all scored young foals and, indirectly, for all animals in the pedigree files. The selection idex is called Total Selection Index (TSI), and accounts for the head size (25%), the temperament (15%), the muscularity (25%), the fore diameters (15%) and rear diameters (20%) EBVs (Mantovani et al., 2005).

Together with the foals, classifiers provide a linear type evaluations (8 traits only: head size, temperament, muscularity, fore diameters, rear diameters, frame size, thorax depth, upper line direction) and a conditions score for the mares.

Liner type classification allows getting field performance and progeny tests.

The third moment of the selection scheme is the admission of the animals to the studbook.

The genetic parameters of equine traits have not been well studied in comparison to other livestock species. This particularly in heavy draught breeds as the IHDH, for which traits related to muscular development (muscularity, fore diameters, rear diameters) have not yet been analysed.

In general it is difficult to compare the evaluations of conformation traits derived from several breeds and accounting for different scoring system, because of serious differences in breeding goals and the scoring approach and methods. However, comparison can be made with cattle hypertrophic breeds that resemble characteristics similar to those observable on heavy draught horses. Sometimes, composite traits can help in comparing different species. For example, Mazza et al. (2014) and Mazza et al. (2015), in Rendena ans Valdostana cattle, were allowed to compare composite muscularity traits, and similarly, the same trait can be recovered also in the IHDH.

The comparisons with beef cattle is more easy, because the breeding goal for meat islikely to the breeding goal of the IHDH. Possible comparison should be therefore be made with studies carried out in Belgian Blue cattle, Piemontese, Chianina (Norris et al., 2008; Forabosco et al., 2004; Veselá et al., 2005; Hanset et al., 1998; Albera et al., 2001; Gutiérrez et al., 2002).

1.4 GENETIC PARAMETERS OF TYPE TRAITS

Selection for one trait produces consequences on other traits. Some gene may indeed effect more than one trait (Pleiotropy), therefore the traits can be genetically correlated. Knowledge of genetic correlations among traits is useful for efficient selection of replacement males and females in any livestock production system. Genetic correlations can be used to predict what is expected to happen to traits other than those used in selection as a result of that selection. In the case of IHDH, it could be worth for the selection purpose, the knowledge of the correlation both within and between type traits scored at different ages. At present, no available literature exists in the correlation between the same traits evaluated at different ages, particularly in horse. The correlations among traits evaluated at 6 and 30 months, would also allow the knowledge on the effect of the selection scheme applied by selecting animals on the basis type traits obtained at younger age, are able to produce any specific effect on the subsequent evaluation obtained later in the life of the IHDH subjects admitted to the studbook. Additionally, this knowledge would be of benefit in case of a shortage of funding that would require the change of the selection scheme and therefore the abandonment of one of the two evaluations carried out on IHDH horses.

1.5 FERTILITY

The impact of negative genetic correlations in livestock traits was disclosed in early 21st century, due to increased productive costs. Concerning cattle, dairy breeding objectives gradually moved their focus from productive traits to functional traits as fertility, longevity and calving ease.

Functional traits aim to improve efficiency not by increasing production but reducing costs, and they are typically related to fitness. The trade-off between muscularity and calving ease in beef cattle moved breeders associations to introduce fitness traits in the aggregate selection indexes.

Cammak et al., (2009), reported that the heritability estimates of reproductive traits in cattle are low because of a large, unexplainable portion of residual variation and the considerable influence of management on many of these measures, and sex.

Mayer et al. (1990), distinguishing between traits pertaining to the lifetime of an individual or repeated over subsequent breeding seasons, showed that the latter traits were generally characterized by a slightly lower heritability.

Studies on horse fertility are few, and a deep analysis of these traits, together with the analysis of their relationship with the productive traits should be of interest, considering the lack of literature on this topic in the horse breeding.

Table 1. Description of the linear type traits evaluated on the Italian Heavy Draught Horse population.

Trait	Evaluation	Descriptor Minimun (1)	Descriptor Maximum (5)
Head size	Evaluate Head and Neck. Head should be not too voluminous and neck should contain a good muscular mass.	Light	Heavy
Temperament	Evaluate the reactivity to stimuli and the movements. Animals are preferred very reactive to environmental stimuli. Lymphatic elements should be underlined and avoided.	Lymphatic	Nevrile
Frame size	Evaluate the rate between trunk development and height. The minimum height at withers should be overcome, such as transverse diameters and thorax depth is the target for a well-shaped animal.	Little	Large
Fleshiness	Evaluate muscular mass at shoulder, back, rump, crouch, and thigh. Subjects are required to present a good muscular mass.	Poor	Excellent
Bone incidence	Evaluate the incidence of the head and the distal parts of the limbs on the whole body. Coarse subjects should be underlined and avoided.	Fine boned	Heavy boned
Thorax depth	Evaluate the height of thorax, i.e., measure the height between withers and sternum.	Little	Large
Fore diameters	Evaluate the width of chest by looking at the width at level of the scapular-humeral articulation.	Narrow	Wide
Rear diameters	Evaluate the width of croup (i.e., at ilium and ischium).	Narrow	Wide
Upper line length	Evaluate the length of back and loins. Short animals are preferred as respect to longer animals.	Short	Long
Upper line direction	Evaluate the direction of the upper line. Kyphosis or lordosis are defects that should be underlined and avoided.	Kyphotic	Inward spine
Rear legs side view	Evaluate the hock articulation. Subjects with sickle or straight defect should be underlined and avoided.	Sickle	Straight
Fore feet	Evaluate the tethers and hooves of the forelegs. Divergent or convergent feet should be underlined and avoided.	Diverging	Converging
Rear feet	Evaluate the tethers and hooves of the rear legs. Divergent or convergent feet should be underlined and avoided.	Diverging	Converging
Hind legs back view	Evaluate the hocks from the back view. Diverging or converging hooks should be underlined and avoided	Diverging	Converging

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CHAPTER 2

AIM OF THE THESIS

The present PhD dissertation, has been designed to evaluate the genetics of productive and fertility traits in the Italian heavy Draught Horse (IHDH) breed.

The studies is based on the collaboration between the University of Padua, Department of Agronomy Food, Natural resources, Animals and Environment, and the National Breeders Association of IHDH (ANACAITPR; Associazione Nazionale Allevatori Cavallo Agricolo da Tito Pesante Rapido) located in Vigasio (Verona), Italy.

In a first step the thesis was aimed at analyzing deeply the genetics of the linear type traits scored in young foals and in adults, aiming at to validate a classification system for the main environmental effect (stud-farm), to analyze the genetic parameters for IHDH population using data obtained at adult age, to evaluate the genetic correlations among traits and between traits recorder at different ages. This part has been structured in three chapters.

A further step of the study was the study of a lifetime fertility trait to be used for the genetic evaluation of reproductive efficiency at population level.

The thesis has been therefore structured in four chapters with the following aims:

i. To study and validate a method to classify the main environmental effect associated to stud farm classification and to estimate genetic parameters (heritability and correlations) for 11 linear type traits scored on young foals;
ii. To study for the first time the genetic parameters of linear type traits scored at adult age (i.e., 30 months) and evaluate the genetic trends obtained from the previous selection process based on index obtained on young foals.
iii. To analyze the genetic correlation between traits scored at different ages (i.e., 6 and 30 months), via Gibbs sample analysis and to calculate the response to selection in different scenarios aiming at evaluating the possible change of the selection scheme based on evaluation of foals with the use of scores obtained later in life on adults.

iv. To study a possible indicator of mares' reproductive career, though the implementation and validation of a lifetime foaling rate variable (LFR) at a given career endpoint, and the analysis of the genetic components of such trait, the genetic trends and the rank correlations based on EBVs obtained with 2 different estimate methods of the LFR and with a LFR arcsin transformed variable, because of the known possible problems due to the use of a ratio variable.

The dissertation ends with a general conclusion based on the results obtained, i.e., the use of genetic parameters obtained at 6 or 30 months of age, and on the possible implementation of the lifetime foaling rate at population level.

CHAPTER 3

GENETICS OF LINEAR TYPE TRAITS CORED ON YOUNG FOALS BELONGING TO THE ITALIAN HEAVY DRAUGHT HORSE BREED

3.1 INTRODUCTION

The Italian Heavy Drought Horse (IHDH) represents today the only autochthonous Italian coldblood breed within the large group of coldblood horses widespread among many European countries (Aberle and Distl, 2004; Mantovani et al., 2005; Saastamoinen and Maenpaa, 2005). The history of IHDH officially starts in 1927, when the first generation of foals born from stallions operating in “selected stud stations” in the northern-east Italian low land was registered. This first generation was obtained by breeding Norfolk-Breton stallions (i.e., known as French Postier Breton; Mantovani et al., 2013) with a group of selected mares that were the results of a long lasting selection carried out since 1861 on local heavy mares crossed with stallions of different heavy breeds (Breton largely, but also Ardennes, Percheron or Belgian horses; Mantovani and Pigozzi, 2003). From this first generation of IHDH, both mares and stallions started to be selected and used in stud stations or sold to individual breeders. In the middle of 1930s the breed accounted for about 250 selected mares and a specific functional test for young stallions aged 3 and 4 years was yearly provided to select Italian born stallions. All the selection efforts were addressed to obtain a heavy strain of horse to be used both in agriculture and field artillery (Mantovani and Pigozzi, 2003; Mantovani et al., 2005). However, after the World War II, when the use for military purposes had lost interest, the IHDH increased its diffusion from the historical north-east area of Italy to other Italian regions as Emilia-Romagna, Abruzzo, Apulia and Sardinia, because of the growing interest for traction and complementary works in agriculture (haymaking, sowing, drawing, etc.; Mantovani et al., 2005). At the end of the 1950’s the breed acquired also its present name (previously it was known as Breton derived horse), to recognize the selection process carried out toward and autonomous standard of heavy horse in Italy. After another progressive decline in numbers during 1960’s and 1970’s due to the mechanization process of agriculture, the IHDH population found a “new deal” during the 1980’s, when its selection goal was re-oriented toward the meat production in addition to the heavy draught (Mantovani et al., 2005). This new selection goal, joined by the novel national breeders association (ANACAITPR; www.anacaitpr.it) sustained by the ministry of Agriculture, produced a further diffusion of the IHDH throughout the national territory, together with a wider selective control allowed by a more capillary recording process established by the new born local breeders associations. As regard the influence of the French Breton on the IHDH, it has to be pointed out that the incidence of stallions imported from France as respect to the total number of approved stallions has been about 18% per year (i.e., 4 stallions per year on an average of 22 yearly approved stallions) from the end of the World War II up to the end of 2003, when any further introduction of the French Breton stallions in the studbook was forbidden. Fluctuation in the number of imported stallions have been observed in this period that covered almost 60 years, but only during the 1980's, that is the time of maximum expansion of the breed, a wider use of the French Breton has been observed.

Starting from 1992, the selection program for the IHDH has been based on a systematic collection of field data carried out yearly through a linear type trait evaluation method involving all new born foals. The linear scoring system was initially developed for cattle breeding purposes aiming at obtaining uniform and accurate sires' evaluation to be used in breeding decisions at farm level when selection goal included type (White, 1974; Thompson et al., 1981). Subsequently, the linear scoring system was applied to a number of commercial livestock, including horses. However, in this specie less homogeneity than in cattle can be observed, due to the multiple and different uses in which horses are involved as respect to other livestock (Saastamoinen and Barrey, 2000). The horse studbooks that have introduced the linear system include saddle horse breed as the Trakehner (Preisinger et al., 1991), the KWPN (Koenen et al., 1995), the Old Kladrub horse (Jakubec et al., 2007), the Belgian warmblood horse (Rustin et al., 2009), the Hanoverian warmblood (Schröde et al., 2010), the Andalusian (Molina et al., 1999; Sánchez et al., 2013), and many other European saddle horses from Germany, Denmark, Finland, Hungary, Ireland, The Netherland, Norway, France, Great Britain, Italy, and Sweden (see for review Koenen et al., 2004). Other studbooks, for which the adoptions of the linear system is known, are of the Shetland Pony (Van Bergen and Van Arendonk, 1993), the Italian Haflinger (Samoré et al., 1997), the Italian Bardigiano Horse (Fioretti et al., 2005), the Iceland horse (Albertsdóttir et al., 2008), the Finnhorse (Schroderus et al., 2010), and the Lusitano horse (Vicente et al., 2014). However, only few examples have been reported for heavy coldblood horse (Czech-Moravian Belgian horse and Silesian Noriker; Vostrý et al., 2009). In addition, the IHDH is one of the few heavy breeds in which linear type traits are scored in foals aged between 2 and 9 months (Mantovani et al., 2005). This in order to carry out a sort of performance testing at population level in which almost all foals are evaluated for 11 linear type traits by trained classifier, 5 of which are combined after the estimates of BVs, in a total merit index (TMI) used as breeding aid for selecting mares and stallions. However, one of the main limits of the system is represented by the reduced number of individuals scored within each stud-farm (Mantovani et al., 2005), producing a too severe data editing when the stud-farm x year x classifier effect, that is the main environmental effect, is introduced within the genetic model for analysing the traits. Some classification systems permitting to recover stud-farms by creating groups of studs have been implemented over years. Groups have been formed alternately considering some available factors (geographical location, housing system, target production, foals vaccination, body condition score of mares).

In this study, type traits collected over a period of 15 years in IHDH breeds were used to: i) validate a unique method for classifying the stud-farm effect, and ii) estimate genetic parameters for linear type traits. Results from this study will be useful to quantify the impact of previous breeding strategies on the morphological characteristics of the IHDH breed.

3.2 MATERIAL AND METHODS

Data Editing

An amount of 17,725 records on type traits routinely recorded on foals since 1992 were preliminarily extracted from the database of the national breeders association of IHDH (ANACAITPR). Records obtained prior to 1997, as well as those belonging to foals with unknown sire, stud-farm, birth date, and birth date of the dam were discarded. Data remaining were further edited in order to classify the main environmental effect on the basis of different grouping of stud-farms. Indeed, because of the small number of records within single levels of stud farms x year x classifier (SYC; average of 2 records), to group the stud farms in environmental units (EU) is necessary to avoid a consistent loss of records (about 26% of SYC includes single foals records). The grouping method considered initially three different complexity levels using the stud book database on stud-farms. At first, each stud farm was assigned to a group by combining its geographical location (north, centre or south of the country) by the type of housing system (stable, feral or semi-feral rearing system). A total of 7 geographical location x housing groups were then identified (i.e., north-feral and south-stable were not identifiable) as grouping method 1 (GRM1). In a second step, a more complex grouping method was obtained by splitting further the 7 GRM1 groups by accounting for 3 possible target production (meat, suckling mares or heavy draught), and for the systematic or unsystematic use of vaccination on new born foals in each stud-farm. This allowed 33 different groups in which stud-farms were classified (grouping method 2; GRM2). Last, as a third step, the 33 GRM2 were further split on the basis of 5 classes of average body condition score (BCS) recorded yearly in each stud-farm on breeding mares (i.e., BCS ≤ 1, 1 > BCS ≤ 1.5, 1.5 > BCS ≤ 2, 2 > BCS ≤ 2.5, BCS > 2.5) as a possible indicator of nutritional management. By combining geographical position, housing, production target, prophylaxis and BCS, a total of 129 groups were obtained as grouping method 3 (GRM3). In regard to the BCS records, it has to be pointed out that these data are obtained yearly on mares at the same time of the type evaluation of foals, since foals stay with the mares at the time of morphological evaluation. The BCS recorded on mares and reported in the foals' evaluation form is based on a 3 point scale system (i.e., 1, 2 or 3; lean, fit or fat, respectively).

From the 3 GRM, 6 different classification systems of the environmental unit for the main environmental effect were obtained as follows:

1. environmental units classified on the basis of GRM1 x year x classifier (EU1; 546 levels);
2. environmental units classified as GRM2 x year x classifier (EU2; 1121 levels);
3. environmental units classified as GRM3 x year x classifier (EU3; 1965 levels);
4. environmental units considered as single stud-farm x year x classifier for all stud farms with at least 2 scored foals born from different stallions (SYC;

1103 levels) or classified as in EU1 (502 levels) if lacking of the previous threshold (EU4; 1605 total levels);

5. environmental units combing part as SYC and part as in EU2 (944 levels) if not in compliance with the threshold of 2 foals scored in SYC from different stallions (EU5; 2047 total levels);
6. environmental units combing part as SYC and part as in EU3 (1543 levels) if not in compliance with the threshold of 2 foals scored in SCY from different stallions (EU6; 2637 total levels).

Aiming at obtaining a homogenous dataset, the remaining data after editing consisted in 11,357 scores of young foals (7,530 females and 3,827 males) aged on average 169 days (i.e., 5.5 months), and accounting for 17,441 subjects in the pedigree files (Table 1).

Models

A set of preliminary ANOVAs (GLM procedure; SAS Inst. Inc., Cary, NC) were carried out to establish which non genetic effects could be taken into account in the final model. In addition to the EU effect, the non-genetic fixed factors that accounted for a significant part of the total variance were the sex, the age of the foals at scoring, and the age of the mares at foals scoring (data not shown). Therefore, the final linear model used for genetic analysis of all type traits can be written as follows.

$$\mathbf{y_{ijklm} = \mu + EU_i + SEX_j + AF_k + AM_l + a_m + e_{ijklm},}$$

where y_{ijklm} is one of the 11 type traits recorded on each foal, μ is the overall mean, EU_i is the effect of the environmental unit i (with a different number of levels depending on the 6 the classification system adopted, as described above), SEX_j is the fixed effect of sex j of each scored foal (j=1 for females, 2 for males), AF_k is the fixed effect of the age of foal at scoring k (9 classes, i.e., ≤ 2 months of age, from 3 to 9 months using 1 month interval and≥10 months of age), AM_l is the fixed effect of the age of the mare at foals' evaluation l (5 classes, i.e., ≤ 4 years, from 5 to 6 years, from 7 to 10 years, from 11 to 13 years and≥14 years of age), a_m is the random additive genetic effect of animal m, and e_{ijklm} the random residual term.

In the most complex analysis carried out, the matrix notation of the abovementioned model can be expressed as follows:

$$\mathbf{y}=\mathbf{X}\boldsymbol{\beta} + \mathbf{W}\mathbf{q} + \mathbf{Z}\mathbf{u} + \mathbf{e},$$

where **y** is an N x 1 vector of observations, **β** is the vector of systematic fixed effects of order p, **q** is the vector of order z of the EU when considered as a random effect (all 6 different models used for validating the classification system of the EU), **u** is the vector of animal effect of order q, and **e** is the vector of residual effects. Furthermore, **X**, **W** and **Z** are the corresponding incidence matrices with the appropriate dimensioni. In the model with the greatest number of random factors, the assumptions about the structure of (co)variance were as follows:

$$\mathrm{Var}\begin{vmatrix} a \\ h \\ e \end{vmatrix} = \begin{vmatrix} A\sigma_a^2 & 0 & 0 \\ 0 & I\sigma_h^2 & 0 \\ 0 & 0 & I\sigma_e^2 \end{vmatrix},$$

where σ^2_a is the additive genetic variance, σ^2_h is the EU variance, σ^2_e is the residual variance, **A** is the numerator relationship matrix, and **I** are identity matrices. On the other hand, the analyses carried out to obtain correlations between type traits were based on the following assumptions on the (co)variance structure:

$$\mathrm{Var}\begin{vmatrix} u \\ e \end{vmatrix} = \begin{vmatrix} G \otimes A & 0 \\ 0 & E \otimes I \end{vmatrix},$$

where **G** and **E** are the (co)variance matrices among traits for the animal and residual effects, respectively; **A** is the additive genetic relationship among animals; **I** is an identity matrix; and ⊗ is the Kronecker product operator.

Analysis

To validate the classification method for the main environmental effect, a set of different single trait analysis was carried out for all 11 type traits. A base model not accounting for the EU effect (BASE) was compared with 6 different models in which the EU obtained from the 6 grouping methods previously described (i.e., EU1-EU6) was included as random effect. The statistics used to compare the different models were the likelihood ratio test (LRT; Visscher; 2006), i.e., a chi-square value calculated as differences between -2 log Likelihood (-2logL) of BASE and -2logL of the 6 different models counting a different EU grouping method

(Visscher; 2006); the percentage squared bias (PSB), proposed by Ali and Schaeffer (1987), and calculated as:

$$PSB=100\ (\mathbf{y} - \hat{\mathbf{y}})'\ (\mathbf{y} - \hat{\mathbf{y}})\ /\ (\mathbf{y}'\ \mathbf{y}),$$

where **y** is a vector of actual and **ŷ** is a vector of predicted values; the mean absolute deviation of residuals (MAD) proposed by Vargas et al. (2000) and obtained from the formula:

$$\Sigma\ |(y - \hat{y})|/\ n,$$

where |y - ŷ| are absolute differences between actual (y) and predicted values (ŷ), respectively, and n is the number of observations; and the standard deviation of residuals obtained from the differences (y – ŷ).

Genetic parameters were obtained by single and bi-trait analyses considering EU6 as main environmental effect and using the AIREML software from the BLUPF90 family (Misztal, 2008). Before running the bi-trait analyses, the single trait analyses addressed the choice of using EU6 as fixed or random effect by comparing models including this effect either as random or fixed by the Akaike Information Criterion (AIC; Akaike, 1974). The standard errors of the heritability (SE_{h^2}) were computed applying the following formula of Lynch and Walsh (1998):

$$SE_{h^2} = h^2 \times \left(\frac{Var(\sigma_a^2)}{(\sigma_a^2)^2} + \frac{Var(\sigma_p^2)}{(\sigma_p^2)^2} - \frac{2Cov(\sigma_a^2,\sigma_p^2)}{\sigma_a^2\sigma_p^2} \right)^{0.5},$$

where h^2 is heritability of a given trait, σ_a^2 and σ_p^2 are the additive genetic and phenotypic variances of the trait, $Var(\sigma_a^2)$, $Var(\sigma_p^2)$ are their respective predicted error variances, and $Cov(\sigma_a^2,\sigma_p^2)$ is the predicted error (co)variance.

Furthermore, standard errors of genetic and phenotypic correlations (SE_r) were computed applying the following formula of Lynch and Walsh (1998):

$$SE_r = r \times \left(\frac{Var(\sigma_1^2)}{4(\sigma_1^2)^2} + \frac{Var(\sigma_2^2)}{4(\sigma_2^2)^2} + \frac{Var(\sigma_{12})}{\sigma_{12}^2} + \frac{2Cov(\sigma_1^2,\sigma_2^2)}{4\sigma_1^2\sigma_2^2} - \frac{2Cov(\sigma_1^2,\sigma_{12})}{2\sigma_1^2\sigma_{12}} - \frac{2Cov(\sigma_{12},\sigma_2^2)}{2\sigma_{12}\sigma_2^2} \right)^{0.5},$$

where r is the correlation between the two traits (genetic or phenotypic), and the other terms are the (co)variances of the traits (1 and 2), and their predicted error (co)variances.

3.3 RESULTS

The means of different scores observed for the 11 linear type traits, reported in Table 2, ranged from 2.77 (for the rear legs side view) to 3.51 (for fleshiness), and standard deviations were in a range between 0.35 (for upper line direction) to 0.64 (for head size/expression). Most of traits analysed showed a mean close to the medium score of 3, although differences among them were observed for the skewness and kurtosis parameters (Table 2). For example, bone incidence, rear legs side view and upper line direction were progressively negatively skewed, indicating few values on the right side, i.e. a greater frequency of data toward fine boned, sickle rear legs and dipped line direction, respectively. On the other hand, upper line length trait showed the greater positive skewness value, that is the evidence of a greater frequency of scores toward a longer line direction of foals' body. Despite the different distributions observed, all traits resulted normally distributed, as indicated by the Kolmogorov-Smirnov and Anderson-Darling tests (data not shown).

Model comparisons

Increasing the complexity of classification for the main environmental unit, i.e., moving from EU1 to EU6, a progressive reduction in the mean and minimum chi-square value of the likelihood ratio test (Table 3), although the reduction resulted still significant as reported in Table 3. However, the more complex classification system adopted, despite a lower but significant minimum LRT, showed a general better fitting as compared to the other models, because of the lower PSB (0.0094), MAD (0.2440) and standard deviation of residues (0.3090; Table 3).

Variance components and heritability

Moving from the previous results, EU6 was alternatively considered as fixed or random factor in single trait models used to estimate variance components (Table 4). The treatment of the EU6 as random effect on average produced smaller values of the additive genetic variance (-0.02; Table 2), and a bit higher estimates of the residual variance (+0.01; Table 4). The EU6 random factor did not absorb a great amount of variance (average σ^2_h=0.04 points^2, that is the 17.5% of the phenotypic variance) in the 11 linear type traits analysed, with few exceptions, i.e., fore diameters (Table 4). An average difference of 0.04 was found in heritability estimates from models alternatively considering EU6 as fixed or random, with lower values in the latter case. More consistent differences moving from the fixed to the random EU6 were observed for the frame size (h^2=0.257 vs. h^2=0.342), the upper line direction (h^2=0.048 vs. h^2=0.149), and rear legs side view (h^2=0.063 vs. h^2=0.174), that showed the greater change of heritability. However, in spite of these substantial changes, the AIC indicated a better model fitting (due to the lower values of AIC; Akaike, 1974) by including EU6 as fixed effect in the model. The mean AIC resulted indeed 13,205 and 14,731 when EU6 was respectively considered as fixed or random (Table 4). Independently from the model, the standard error of the heritability (SE_{h2}) resulted generally low, ranging between 0.016 and 0.028 (Table 4). The linear type traits that showed the greater SE_{h2} were the head size/expression, the fleshiness, the fore diameters, and the read diameters (Table 4)

The heritability obtained accounting the fixed EU6 resulted of in general of medium magnitude ranging from 0.048 to 0.355 (Table 4). The most heritable traits were fleshiness (h^2=0.355), head size/expression (h^2=0.348), the fore diameters (h^2=0.347), and the rear diameters (h^2=0.300), while the lowest values for upper line length (h^2=0.097), rear legs side view (h^2=0.063), and upper line direction (h^2=0.048). On the other hand, temperament and frame size showed moderate-low h^2 (0.198 and 0.257, respectively). When the EU6 was accounted as random effect the heritability resulted on average increased, but changes affected mainly trait with low or very low heritability estimates obtained with the alternative fixed EU6.

Genetic and phenotypic correlations

Estimates of genetic correlations (r_g) between traits obtained under a model considering EU6 as fixed effect ranged from -0.600 to 0.929 (Table 5).

The most negative genetic correlations involved the bone incidence. Indeed, highly negative correlations were observed between bone incidence and the head size/expression (r_g=-0.600±0.074), bone incidence and temperament (r_g=-0.566±0.093), and between bone incidence and rear legs side view (r_g=-0.510±0.141). Medium negative genetic correlations were also observed between the upper line length and the upper line directions (r_g=-0.378±0.191) and between the upper line length and the rear legs side view (r_g=-0.294±0.170). The upper line length resulted negatively correlated, although at a lower magnitude, to the head size expression (r_g=-0.183±0.102), and to the fleshiness (r_g=-0.134±0.103) from the genetic point of view. The greatest genetic correlations were observed for fleshiness and diameters (fore and rear) traits (Table 5). Fleshiness resulted strongly correlated to both the fore diameters (r_g=0.833±0.031), and the rear diameters (r_g=0.929±0.024), that showed between them a strong inter-relationship, i.e. r_g of 0.878±0.029. Also the thorax depth showed a medium high genetic correlation with fore and rear diameters (r_g=0.657±0.059 and r_g=0.741±0.055, respectively) as well as with the fleshiness (r_g=0.694±0.056), and with the frame size (r_g=0.551±0.071). The frame size showed medium high genetic correlations with fore diameters (r_g=0.672±0.049), rear diameters (r_g=0.538±0.057) and fleshiness (r_g=0.463±0.060), in decreasing order. Medium high genetic correlation was also obtained for head size/expression and temperament (r_g=0.562±0.063).

In general, phenotypic correlations (r_p) resulted lower than the respective genetic correlations and, with few exceptions, maintained the same sign than the latter ones (Table 5). As for the genetic ones, the greatest phenotypic correlations were found among fleshiness, fore, and rear diameters, showing a r_p≥0.50. Phenotypic correlations between 0.30 and 0.50 were observed for thorax depth with frame size, fleshiness, fore and rear diameters, or for frame size compared to fore and read diameters. Again, the lowest values of phenotypic correlations were those observed between the bone incidence and the head size/expression (r_p=-0.292±0.011), or between the first trait and the temperament (r_p=-0.180±0.011).

The approximate standard errors (SE_{rg}) obtained for genetic correlations ranged from 0.024 to 0.216, although the SE_{rg} of greater magnitude as compare to the r_g estimates were in most cases those involving the upper line (length and direction) and the rear legs side view, i.e., the traits that presented greater distribution asymmetry. On the other hand, SE_{rp} obtained for the r_p estimates resulted very low, i.e., between 0.008 and 0.013.

3.4 DISCUSSION

Model comparisons

This study has proved that the increase complexity of the classification system for the environmental unit (EU) including the stud-farms and other factors such as the housing system and the geographical location can increase the goodness of fit as compared to a base model or to less complex models. The problem of accounting for stud-farms information when the number of horses within herd is low has been barely considered in horse breeding and genetics literature. In their work about Italian Haflinger horse, Samoré et al. (1997), considered the herd-group, an environmental unit including herds grouped by geographical area, management and birth year. That permitted to retain the herds with few animals for genetic analyses. This method was adopted in the genetic evaluation of other horse breeds with small herds, e.g. in Bardigiano horse (Fioretti et al., 2005). A similar approach was applied elsewhere, e.g. by Sánchez et al. (2013) that grouped the birth stud-farms of Pura Raza Español horse by geographical region. Likewise, Druml et al. (2008) analysed data of Noriker light draught horse by introducing the fixed effect of the evaluation day as a sort of region effect, since animals from each of the seven Austrian geographic regions of the study were scored in the same day. Some other methods for grouping small herds were proposed in other breeds such as cattle: e.g., Vasconcelos et al. (2008) applied a clustering procedure based on the Ward's minimum variance (CLUSTER procedure; SAS Inst. Inc., Cary, NC) and grouped small herds by 14 indicators of production environments (herd size, average age at first calving, production level…). In a study on Slovenian dairy cattle, Jenko et al. (2013) grouped some small herds considering the number of animals inside the herd, the agricultural and climatic area. The geographical region, the production level and the management have been generally considered in animal breeding as main criteria for establishing environmental units of herds. The possibility to group small stud-farms by province was initially studied in IHDH genetic evaluations (ANACAITPR, *personal communication*), but better results in terms of individual breeding values were obtained by grouping farms by geographical region and management as in Samoré et al. (1997), and also considering the farm production objective of meat or draught, the foal prophylaxis, and the mean BCS of mares (Mantovani et al., 2005), as in GMR3 of the current study.

Moving from the background of genetic evaluations routinely performed in IHDH, this study aimed to validate the classification method for the main environmental effect, the EU, by evaluating an increasing number of indicators and the possibility to use only groups of stud-farms or a mixture of groups and single stud-farms. The likelihood ratio test has permitted to evaluate how much each EU1-EU6 effect is able to explain phenotypic variation more than a model without the environmental effect (BASE). The LRT statistics is commonly applied in hypotheses testing when maximum likelihood is used to estimate genetic and environmental (co)variances, since it has desirable asymptotic properties e.g. following a χ^2 distribution with degrees of freedom equal to the different number of parameters of models tested (Visscher, 2006). The amount of unexplained variation that a model with more parameters is able to describe respect to a reduced model (i.e., the model deviance) corresponds to this χ^2 distribution (Burnham and Anderson, 1998). The LRT is commonly used in model comparisons studies, including in horse breeding (e.g. in German trotters; Bugislaus et al., 2006). Each model including a EU1-EU6 effect significantly reported a better fitting than BASE model, hence demonstrating the importance of including the main environmental component in genetic analysis. The choice of the EU6 model despite the lowest value of LRT is due to the better model fitting after analysis of residuals, i.e. PSB and MAD statistics. Firstly developed to fit lactation curves of dairy cattle in regression models, both statistics permits to assess the difference between the actual and the predicted phenotypic variation (Ali and Schaeffer, 1987; Vargas et al., 2000). Similar criteria based on residuals analysis have been considered also in horse breeding literature, e.g. the mean square error of prediction (MSEP), used for conformation and riding ability in Icelandic horses (Albertsdóttir et al., 2012).

The last model fitting criterion considered in this study, i.e., the AIC, has permitted to choose whether introducing the EU6 effect as fixed or random because this statistics allows to compare non-nested models by correcting the likelihood of each model for the number of parameters included (Akaike, 1974). The goal of AIC is to identify the model that is most plausibly generated by data, and a statistically significant difference between models is pretty arbitrarily proposed (e.g. an AIC difference of 4-7 points roughly corresponds to a 95% confidence; Burnham and Anderson, 1998). The large differences in AIC between models including EU either as fixed or random suggest a better fitting for models with fixed EU. A previous study on linear type traits in IHDH found a better fitting for the model treating the main environmental effect as random (Mantovani et al., 2010), but the analysis did not consider the classifier effect, included in the present study as combination stud x year x classifier (SYC, see Materials and Methods). In addition to the preliminary ANOVA on fixed effects for this study, a pilot study on non-genetic variance components in IHDH linear type traits (Folla and Mantovani,

2013) has found a statistical significance of the SYC effect codified as EU6 for all the linear traits under investigation.

Stud-farm/geographic region and classifier effects, often joined to the evaluation year, have been introduced together in a number of studies on linear type traits in horses, e.g. in Dutch warmblood horse evaluation (Koenen et al., 1995; Ducro et al., 2007), in which the place x date and classifier was considered, in Italian Haflinger (Samoré et al., 1997), accounting for herd group x birth-year and classifier x evaluation-year, and in Pura Raza Español horse (Sánchez et al., 2013), evaluating the geographic region, and the classifier x recording event. The combination Herd x year x classifier, not included in horse breeding studies excluding the present (SYC effect), may be also found in type traits evaluation of cattle (e.g. Uribe et al., 2000).

Genetic evaluation for linear type traits of some horse breeds, e.g. Andalusian horse (Molina et al., 1999), Bardigiano (Fioretti et al., 2005), Noriker (Druml et al. 2008) did not considered the classifier effect despite the classifiers' subjectivity in scoring (Preisinger et al., 1991). An interesting case is the Old Kladrub horse, evaluated by just one classifier from 1995 to 2004 (Jakubec et al., 2007).

Heritability estimates

A number of traits are routinely recorded in horse linear type evaluation, and the kind of traits scored for each breed mainly depends on the selection purpose. It is generally difficult to compare evaluations of conformation traits from different breeds, due to major differences in breeding goals and scoring systems (Druml et al., 2008). Some traits are evaluated in both light and draught breeds, such as the head and the upper line, whereas other are typical of light (e.g. gait correctness, heels) or draught (e.g. frame size, diameters) ones (Koenen et al., 1995; Mantovani et al., 2005; Vostrý et al., 2009; Sánchez et al., 2013).

The linear type traits considered in IHDH are commonly scored in draught breeds with exception of temperament, measured in in few light breeds as the Haflinger and the Andalusian horse (Samoré et al. 1997; Molina et al., 1999). Temperament in IHDH is measured with the aim to obtain lively subjects with good movement skills, and to prevent the onset of lymphatism (ANACAITPR; www.anacaitpr.it). The trait summarizes the impulsion to movement, the reaction to environmental stimuli, and extension and regularity of steps. Together with head size/expression, fore and rear diameters and fleshiness, temperament is one of the traits scored in six-months young foals and included within the selection index (Total Merit Index, TMI; Mantovani et al., 2005) of the breed.

In the present study, temperament reported a medium heritability (h^2=0.20), higher than those found by Samoré et al. (1997; h^2=0.06) in Haflinger, and by Molina et al. (1999; h^2=0.06) in Andalusian, but similar to the one found for the trait called "movement" by Druml et al. (2008; h^2=0.20) and by Molina et al. (1999; h^2=0.15). Definition of movement in these authors is similar to the one of temperament in IHDH, i.e. agility, pace and extension of steps, quality of trot. Conversely, temperament is described by Molina et al. (1999) as ease of response to handling and gentleness.

About the other traits included in TMI, the head size/expression, evaluating in IHDH the animal posture and the head volume, reported in the present study an heritability of 0.35. Samoré et al (1997), averaging the values of head size and head expression in Haflinger, found an h^2=0.24, while Molina et al. (1999), reported a value of h^2=0.23 in Andalusian horse, and a value of h^2=0.21 was reported by Koene et al. (1995) in Dutch Warmblood. Rustin et al. (2009) in Belgian Warmblood horse, found a highest value of h^2=0.45. Again, Druml et al. (2008) found different heritabilities for the linear score (h^2=0.11) and for the morphometric measures (average h^2=0.37) of the head in the Noriker horse, higher than the morphometric estimates for head (average h^2=0.15) in Lipizzan horse (Zechner et al. 2001). The reason of differences among studies are probably due to the breed, the purpose of selection, and the method of evaluations (in the Haflinger, Andalusian and Noriker a scale from 1 to 10 is used, the Belgian warmblood evaluation uses a scale from -20 to 20, while in Lipizzan only body measurements are used).

The scoring of diameters in IHDH is aimed to evaluate the width of the anterior and posterior quarters, including thorax and croup width (ANACAITPR, www.anacaitpr.it). In this study the heritability of fore diameters was 0.35, higher than reported for Noriker by Druml et al. (2008; h^2=0.16), and for Haflinger by Samoré et al. (1997), that presented a h^2 value of 0.11. Molina et al. (1999) estimated an heritability of 0.40 for Andalusian horse. Zechner et al. (2001) reported a value of 0.31 for the morphological measure of chest width, whereas

Miserani et al. (2002), found an higher value (h^2=0.51) for the chest width score in Pantaneiro horse.

The rear diameters heritability (h^2=0.30) in the IHDH is higher than those found by Samoré et al (1997; h^2=0.30 for croup width), Druml et al (2008; h^2=0.20 for rear quarter), Molina et al (1999; h^2 around zero for croup and tail), while similar values were found by Zechner et al (2001; h^2=0.31 for hips width), Koenen et al. (1995; h^2=0.28 for shape of croup) and Rustin et al. (2009; h^2=0.30 for shape of croup) respectively in Lipizzian horse and Dutch and Belgian warmblood. An higher value of 0.52 was found by Miserani et al. (2002) in Pantaneiro horse.

As regards fleshiness, that considers the muscular development of withers, back, loin, rump, thigh, and buttock (ANACAITPR, www.anacaitpr.it), the estimates obtained in this study (h^2=0.35) are difficult to compare with other horse breeds, due to the lack of similar studies. Some comparison can be made with cattle hypertrophic breeds. Looking at literature on this topic, heritability estimates reported e.g. by Albera et al. (2001) on muscularity in Piemontese young bulls ranged from 0.26 to 0.55. In the study of Norris et al (2008), muscularity showed a heritability of 0.35 in Charolaise breed, and an heritability of 0.30 was found for fore and rear diameter.

The heritability of temperament, fore and rear diameters, and fleshiness of this study are lower than reported by Mantovani et al. (2010) for the same traits in IHDH, as well as the standard errors (average SE_{h2}=0.02). The difference may be due to the different model (not including classifier) and number of foals analysed.

Phenotypic and genetic correlations

Some considerations can be carried out about the genetic correlations among linear traits considered in this study. Genetic correlations between many pairs of traits were low and sometimes close to zero, i.e. r_g between -0.30 and 0.30, suggesting virtually independence of these traits and a low correlated indirect selection. Some high ($r_g \geq 0.60$) correlations were also obtained for traits measuring close aspects of the body structure (see further).

Specifically, head size/expression reported low r_g with all traits excluding temperament, bone incidence and rear legs side view (Table 5). A positive genetic relationship among a good head shape, a thin bone structure, a correctness in upper line direction and legs posture, and a regularity in steps and gaits (this latter scored within the temperament) has been found (Table 5). In IHDH selection, horses with a not too voluminous head, and a not too short neck, but with good development of muscular masses, are preferred. A short upper line and a thin bone structure are also desired in IHDH selection, the latter to avoid a great bone incidence within the carcass (ANACAITPR, www.anacaitpr.it). The genetic correlation between bone incidence and fleshiness is around zero (Table 5) because to avoid possible overlaps classifiers are called to score bone incidence in relation to muscle masses and not as an absolute value (ANACAITPR, www.anacaitpr.it).

The moderate-high (r_g from 0.46 to 0.93; Table 5) genetic relationships that have been found among frame size, fleshiness, thorax depth and fore and rear diameters are both because these traits regard close aspects of the same body region (e.g., thorax depth and flashiness), and also because frame size is a linear combination of thorax depth, fore and rear diameters, and stature (the only trait not already individually scored). The highest genetic correlations, varying from 0.83 to 0.93 and found among fleshiness, rear and fore diameters, reflect the fact that large chest and rump offer a wider space for the muscle masses. The considerations done at genetic level may be applied at phenotypic level as well, as shown by the phenotypic correlations lower than the corresponding r_g but of the same sign (Table 5).

A positive genetic correlation between head size/expression and temperament (r_g=0.56 in the present study) was also found by Druml et al (2008; r_g=0.66), whereas Albertsdóttir et al (2008) estimated a lower value (r_g=0.11) between head and general impression. Viklund et al. (2008) found in the Swedish warmblood foals a genetic correlation of 0.36 between the traits head-neck- body and correctness of legs, that is close to the value (r_g=0.38) reported in the present study. Different values were found in literature for the genetic correlations between head size and both fore and rear diameters, respectively of r_g=0.17 and r_g=0.09: Bakhtiari et al. (2009), found a genetic correlation of 0.52 between morphometric measures of head and chest width, corresponding with fore diameters, in Iranian Thoroughbred horse, and a similar value was found by Van Bergen and Van Arendonk (1993) in Shetland pony. Vicente et al (2014) found a r_g=0.54 between head-neck and croup (corresponding with rear diameters) in Lusitano horse, and r_g=0.081 with chest.

Drum et al (2008) found in Noriker horse r_g values higher than in IHDH between head and fore (r_g=0.74) and rear diameters (r_g=0.58). Similarly, Molina et al (1999) found a correlation of r_g=0.69 between head and croup in Andalusian horse. Medium-high correlations were found among diameters and fleshiness, as well as in IHDH: Koenen et al. (1995) found a r_g=0.70 between muscularity of haunches and the shape of croup in Dutch warmblood horse, while Druml et al. (2008) reported a r_g=0.46 between rear quarter and chest circumference. Molina et al. (1999) reported a value of 0.35 between genetic correlation of chest-thorax and croup-tail. Vicente et al (2014) reported a value of 0.52 between chest and croup. Looking at genetic correlations in bovines, and involving fleshiness, high r_g were found between muscularity, fore and rear diameters: e.g., Norris et al. (2008) reported genetic correlations of 0.85 and 0.93 between muscularity and respectively fore and rear diameters, as well as between fore and rear diameters a rg=0.88 was found.

3.5 CONCLUSIONS

The main environmental effect, represented by the stud-farms, has shown a great incidence on the variability of linear type trait evaluations performed in Italian Heavy Draught Horse at 6 months of age. Aiming to retain the most information as possible in genetic analysis, the most effective solution is to include small farms within environmental units (EU) considering groups of stud-farms x year x classifier. Three different grouping methods (GRM) have been considered in the study, and the possibility to include within groups either all studs, or only small studs with less than 2 scored foals from different stallions has been evaluated. The best way to group stud-farms is to use information about housing system, geographical location, target production, use of vaccination, body condition score of mares. The best solution for genetic analyses is to apply this grouping method to the small studs and to include the other studs as single environmental cells, and then consider the such obtained stud-farms x year x classifier effect as fixed.

About genetic parameters estimated, medium heritabilities have been found for traits involved in the muscularity development, such as fleshiness, fore and rear diameters, thorax depth and frame size, and medium-high correlations have been found among them. The genetics correlations among fleshiness, fore and rear diameters were particularly high (r_g ranged from 0.83 to 0.93), and corresponding phenotypic correlations were high as well (r_p ranged from 0.50 to 0.57). A regularity in gaits, a not too big head, thin bone structure and correctness in upper line and rear legs have also shown positive genetic correlations, and they represent appreciable characteristics in IHDH breeding.

All traits involved in Total Merit Index (head size, temperament, muscularity, fore diameters, rear diameters) have reported a medium-high heritability (r_g ranged from 0.20 to 0.35). The goal of selection in IHDH is to obtain an animal with a good muscular mass, but elegant and brilliant in the movements.

Further increases of genetic potential are surely desirable, above all because a progressive detriment in genetic variance, both due to selection and to the inbreeding, has been experiencing in the new generations. However, the increase of artificial insemination practice, actually used only for 10% of inseminations, could support a further improvement of the breed.

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Table 1. *Descriptive statistics on the final data set retained for the analyses carried out*

Item	Value
Records	11,357
Females	7,530
Males	3,827
Animals in the pedigree file	17,441
Sires with scored foals	835
Dams with scored foals	4,697
Age of Foals, d	169.2
Age of Mares at Foals' evaluation, yr	4.51

Table 2. *Descriptors for the 11 linear type traits recorded on Italian Heavy Draught Horse and descriptive statistics (mean±standard deviation; skewness and kurtosis) obtained on 11,357 foals scored between 1997 and 2012*

	Descriptor range[1]				
Trait	Min.(1 score)	Max. (5 score)	Mean±sd	Skewness	Kurtosis
Head size/Expression	Heavy	Light	3.17±0.64	-0.11	0.01
Temperament	Lymphatic	Spirit	3.35±0.52	0.16	0.09
Frame size	Little	Large	3.32±0.59	-0.25	-0.11
Fleshiness	Poor	Excellent	3.51±0.55	-0.47	0.02
Bone Incidence	Fine boned	Heavy boned	2.72±0.45	-0.77	-0.25
Thorax Depth	Little	Large	3.46±0.50	-0.22	-0.49
Fore Diameters	Narrow	Broad	3.06±0.64	-0.18	-0.16
Rear Diameters	Narrow	Broad	3.42±0.56	-0.30	-0.26
Upper line length	Short	Long	3.20±0.43	0.73	0.95
Upper line direction	Dipped	Arched	2.87±0.35	-1.99	3.16
Rear legs side view	Sickle	Straight	2.77±0.41	-1.22	0.73

[1] Scale from 1 to 5 considering also half points for a total of 9 classes.

Table 3. *Results of model comparison between a base model not accounting for the main environmental unit and models that grouped the stud-farms in different methods (EU1-EU6[1])*

Model	Levels	LRT[2]			PSB[4]	MAD[5]	residuals' SD[6]
		Mean	Minimum	P<CHI[3]			
Base	-	-	-	-	0.0121	0.2811	0.3501
EU1	546	956.6	410.9	<0.001	0.0113	0.2663	0.3385
EU2	1,121	832.1	339.1	<0.001	0.0108	0.2599	0.3301
EU3	1,965	691.9	277.9	<0.001	0.0101	0.2516	0.3191
EU4	1,605	770.6	331.9	<0.001	0.0104	0.2557	0.3243
EU5	2,047	698.7	279.0	<0.001	0.0099	0.2502	0.3173
EU6	2,637	616.4	245.5	<0.001	0.0094	0.2440	0.3090

[1] EU1 considers the grouping of stud farms on the basis of their geographical location and type of housing system (GR1) by year-classifier; EU2 considers stud farms grouped as in GR1 but further divided by accounting for 3 possible target production (meat, suckling mares or heavy draught), and for the systematic or unsystematic use of vaccination on new born foals (GR2) by year-classifier; EU3 considers GR2 further split on the basis of 5 classes of average body condition score recorded at time of foal scoring in each stud-farm on all breeding mares (GR3) by year-classifier; EU4 considers a mixture of single stud farm by year-classifier (SYC) for all stud farm with at least 2 scored foals born from different stallions, otherwise grouped as in GR1 by year-classifier; EU5 considers a mixture between SYC where possible and the grouping of stud farms as in GR2 by year-classifier; EU6 considers a mixture between SYC where possible and the grouping of stud farms as in GR3 by year-classifier.

[2] LRT=Likelihood ratio test (mean and minimum chi-square value for 11 type traits), obtained as differences between -2 log Likelihood (logL) of base model and -2logL of the model in each row (Visscher; 2006).

[3] P<CHI=Probability for the minimum Chi-square value obtained as LRT test with 1 degree of freedom (Visscher; 2006).

[4] PSB=percentage squared bias, calculated as $100\ (\mathbf{y} - \hat{\mathbf{y}})\ '\ (\mathbf{y} - \hat{\mathbf{y}})\ /\ (\mathbf{y}'\ \mathbf{y})$, where $\mathbf{y}$ is a vector of actuals and $\hat{\mathbf{y}}$ is a vector of predicted values (Ali and Schaeffer, 1987).

[5] MAD=Mean absolute deviation of residuals, that is □(y - ŷ)/ n, where y and ŷ are actual and predicted values, respectively, and n is the number of observations (Vargas et al., 2000).

[6] residuals' SD=standard deviation of residuals obtained as y – ŷ, where y and ŷ are actual and predicted values, respectively.

Table 4. *Variance components*[1]*, heritability (h^2), standard error of heritability (SE h^2) and Akaike Information Criterion (AIC; Akaike, 1974), resulting from single trait analysis of type traits accounting for the environmental unit classified as EU6*[2]*and considered as fixed or random effect*

	Fixed EU6					Random EU6					
Trait	σ^2_a	σ^2_e	h^2	SE h^2	AIC	σ^2_h	σ^2_a	σ^2_e	h^2	SE h^2	AIC
Head size/Expression	0.117	0.219	0.348	0.028	17,788	0.037	0.144	0.204	0.375	0.021	20,057
Temperament	0.043	0.173	0.198	0.024	14,357	0.042	0.060	0.164	0.225	0.018	16,115
Frame size	0.065	0.187	0.257	0.026	15,560	0.047	0.108	0.162	0.342	0.020	17,679
Fleshiness	0.085	0.154	0.355	0.028	14,799	0.040	0.098	0.147	0.345	0.020	16,531
Bone Incidence	0.019	0.140	0.120	0.022	11,878	0.034	0.028	0.134	0.144	0.017	12,866
Thorax Depth	0.033	0.153	0.176	0.024	13,121	0.047	0.043	0.146	0.181	0.017	14,638
Fore Diameters	0.094	0.178	0.347	0.027	15,978	0.075	0.126	0.162	0.348	0.020	18,745
Rear Diameters	0.073	0.170	0.300	0.027	15,124	0.046	0.093	0.159	0.313	0.020	17,067
Upper line length	0.015	0.137	0.097	0.021	11,557	0.028	0.030	0.128	0.162	0.018	12,322
Upper line direction	0.004	0.070	0.048	0.018	5,338	0.034	0.017	0.061	0.149	0.016	5,496
Rear legs side view	0.008	0.115	0.063	0.018	9,757	0.033	0.028	0.102	0.174	0.018	10,522

[2] Variance components: σ^2_a=additive genetic; σ^2_h=environmental; σ^2_e=random residual.

[1] EU6 treated as fixed was chosen as best method to account for the for the main environmental units and consisted in a classification of stud farms on the basis of a mixture between single stud farm by year-classifier (SYC) for all stud farm with at least 2 scored foals born from different stallions, otherwise in a grouping the remaining stud farms on the basis of their geographical location, type of housing system, target production (meat, suckling mares or heavy draught), systematic or unsystematic use of vaccination on new born foals, and accounting for 5 classes of mares' average body condition score. Groups of stud farms were then accounted as group-year-classifier.

***Table 5.** Estimates of phenotypic (below diagonal) and genetic (above diagonal) correlations and standard errors (values within brackets) obtained considering fixed EU6[1] as main environmental unit and treated as fixed effect*

Trait	Hs/E	Te	Fs	Fl	Bi	Td	Fd	Rd	Ul	Ud	Rl
Head Size/Expression (Hs/E)	-	0.562	0.048	0.137	-0.600	0.044	0.171	0.090	-0.183	0.160	0.377
		(0.063)	(0.071)	(0.062)	(0.074)	(0.081)	(0.063)	(0.067)	(0.102)	(0.142)	(0.114)
Temperament (Te)	0.290	-	0.294	0.148	-0.566	0.182	0.242	0.156	0.043	0.406	0.340
	(0.011)		(0.082)	(0.076)	(0.093)	(0.096)	(0.075)	(0.080)	(0.122)	(0.162)	(0.128)
Frame size (Fs)	0.086	0.180	-	0.463	0.321	0.551	0.672	0.538	0.379	0.171	0.065
	(0.012)	(0.012)		(0.060)	(0.098)	(0.071)	(0.049)	(0.057)	0.103)	(0.158)	(0.132)
Fleshiness (Fl)	0.144	0.106	0.298	-	0.031	0.694	0.833	0.929	-0.134	-0.028	-0.052
	(0.013)	(0.012)	(0.011)		(0.095)	(0.056)	(0.031)	(0.024)	(0.103)	(0.136)	(0.121)
Bone incidence (Bi)	-0.292	-0.180	0.024	-0.101	-	0.191	0.137	0.131	0.250	-0.019	-0.510
	(0.011)	(0.011)	(0.012)	(0.012)		(0.115)	(0.094)	(0.100)	(0.141)	(0.191)	(0.141)
Thorax depth (Td)	0.072	0.057	0.329	0.341	0.034	-	0.657	0.741	-0.016	-0.020	0.123
	(0.012)	(0.012)	(0.011)	(0.011)	(0.012)		(0.059)	(0.055)	(0.127)	(0.167)	(0.143)
Fore diameters (Fd)	0.130	0.116	0.410	0.497	-0.024	0.350	-	0.878	0.159	0.095	0.042
	(0.013)	(0.012)	(0.010)	(0.009)	(0.012)	(0.011)		(0.029)	(0.101)	(0.141)	(0.120)
Rear diameters (Rd)	0.108	0.117	0.417	0.569	-0.036	0.390	0.552	-	0.012	0.127	0.136
	(0.013)	(0.012)	(0.010)	(0.008)	(0.012)	(0.010)	(0.008)		(0.109)	(0.151)	(0.125)
Upper line length (Ul)	-0.073	0.016	0.131	-0.064	0.052	-0.053	0.019	0.009	-	-0.378	-0.294
	(0.012)	(0.012)	(0.012)	(0.012)	(0.011)	(0.011)	(0.012)	(0.012)		(0.191)	(0.170)
Upper line direction (Ud)	0.042	0.023	0.020	-0.027	-0.015	-0.015	-0.018	-0.032	-0.106	-	0.360
	(0.012)	(0.011)	(0.011)	(0.012)	(0.011)	(0.011)	(0.012)	(0.012)	(0.011)		(0.216)
Rear legs side view (Rl)	0.079	0.109	0.012	-0.007	-0.075	-0.015	-0.004	-0.004	-0.055	0.055	-
	(0.012)	(0.011)	(0.012)	(0.012)	(0.011)	(0.011)	(0.012)	(0.012)	(0.011)	(0.011)	

[1] EU6 treated as fixed was chosen as best method to account for the for the main environmental units and consisted in a classification of stud farms on the basis of a mixture between single stud farm by year-classifier (SYC) for all stud farm with at least 2 scored foals born from different stallions, otherwise in a grouping the remaining stud farms on the basis of their geographical location, type of housing system, target production (meat, suckling mares or heavy draught), systematic or unsystematic use of vaccination on new born foals, and accounting for 5 classes of mares' average body condition score. Groups of stud farms were then accounted as group-year-classifier.

CHAPTER 4

GENETICS OF LINEAR TYPE TRAITS SCORED ON ADULTS BELONGING TO THE ITALIAN HEAVY DRAUGHT HORSE BREED

4.1 INTRODUCTION

The goal of breeding organisations and individual breeders is to improve the genetic value of animals over generations. This objective is facilitated by the assessment of breeding stock with valuable characteristics to the breeding program. Conformation evaluation regards aspects as the morphology and movement, and it is an important aspect of this assessment process in a number of livestock species including pigs, sheep, cattle and horse (Breen et al., 2009). Traits may be measured or scored, depending on the breeding management of the breed. In the first case, traits are measured objectively through e.g. a meter, in the latter a trained evaluator provides a subjective judgement about the correctness of the target trait (Saastamoinen and Barrey, 2000). In some horse breeds a linear evaluation system developed in dairy cattle (Thomson, 1981) was introduced in the last decade of 20th century (e.g., in Dutch Warmblood, Koenen et al., 1995; in Italian Halfinger, Samoré et al., 1997). Under this system, extreme scores correspond to the biological extremes of the trait, and individual scores lie between these extremes. Heritabilities of linear traits are consistent with the ones obtained through a subjectively scoring, as reviewed in Saastamoinen and Barrey (2000) for various breeds. The current conformation of a horse is the result of both natural and breeders' selections, and the traits evaluated for each horse population depend on the different breeding purposes of the breed. While head, neck and shoulders are evaluated almost in all horse breeds, horses bred for racing and riding performances are used to score the regularity of gaits, the walk and the trot, whereas draught horses put great attention on fore and rear quarters (Saastamoinen and Barrey, 2000; Druml et al., 2008). Meat horses are also evaluated for traits related to meat production, such as thorax depth or fleshiness (e.g. Fioretti et al., 2005; Gomez et al., 2005), the latter typically considered in meat cattle and only evaluated in Italian Heavy Draught Horse (IHDH; Mantovani et al., 2005; Folla and Mantovani, 2013).

The achievement of the conformation standards typical of the breed is a first requirement for the admission to the stud book. In the breeding programme of e.g. the Royal Dutch Warmblood stud book, two different types of traits are recorded at stud book entry: descriptive (as walk and trot, scored linearily) and subjective (overall conformation and movement, valued from very bad to excellent). Evaluation is used to occur at 3-7 years of age, and involves most of the young horses of the breed

(Ducro et al., 2007). Various methods of evaluation are used in different breeds (some examples are reported in Table 1), and most of them are evaluated one time in their life.

In the IHDH, linear evaluation is used, and for the stud book entry animals are evaluated two times during life, at about 6 (foals) and 30 (adults) months of age. The first evaluation concerns the access to the Foals Register, the latter the inscription at the Stallions and Mares Register (ANACAITPR, 2010). The linear type evaluation in IHDH (Mantovani et al., 2005) involves in the young age the scoring of 11 traits using a scale from 1 to 5 (based on biological scale) that can be assigned to the three main groups of general aspect, trunk, and legs. In adult age three traits about the correctness of feet and legs are added, as well as an additional overall score of conformation (Folla et al., 2013).

The selection scheme set up for the IHDH is based on the genetic evaluation for linear type traits since 1992 (ANACAITPR, 2010). Genetic improvement in IHDH has the dual purpose of meat and draught: although the main goal selection is meat production, in recent years an increasing interest for the rapid draught has occurred, and it has implied the use of horse in team races and in agricultural works in the organic farms (Mantovani et al., 2005). Genetic evaluation is based on the linear type traits obtained on 6-months-young foals, five of them weighted summed in a Total Merit Index (TMI) indicating the selective value of subject (Mantovani et al., 2005; 2010b).

The TMI and the morphological evaluation are main criteria for the stud book: male foals to be admitted to stud book need at least 3 generations of known ancestors, a minimum pedigree index for TMI of 100 (the index is setted with mean at 100 and standard deviation at 10; Mantovani et al., 2005) and a minimum final morphological score of “good”, while female foals require just a minimum final morphological score of “fair”. Stallions have to be previously registered in the stud book as foals and with a breeding value for TMI at least of 100 if aged 3 years or more, and a minimum TMI of 110 if aged 2 years. In the mares category there are just information about the number of sons calved and evaluated (Mantovani et al., 2005).

Traits scored on 30-months-animals are not directly used for genetic improvement, but they are mandatory for the final admission of candidate stallions and mares to the stud book (Folla et al., 2013). The estimation of genetic parameters in linear type traits scored at adult age has been not performed yet, although in IHDH genetic improvement the use of traits scored at 30 months, instead of at 6 months, could be interesting in terms of cost reduction (about 450 animals/year are evaluated vs. 800 foals/year) due to the current shortage of funding to breeders associations (Folla et al., 2013). Traits scored on 6-months foals at performance testing allow to speed up genetic gain for selected traits due to the younger animals used, but also 30-months scoring could allow a sound genetic improvement. Furthermore, 30 months is closer to the ages at which the admission to stud book is used to be inspected in horse breeds (e.g., in Andalusian horse; Molina et al., 1999).

Following these considerations, this study aimed to estimate genetic parameters of liner type traits in Italian Heavy Draught Horse evaluated at the adult age of about 30 months, to assess the genetic correlations among traits, and estimate the genetic trends. In terms of genetic improvement of the breed, this is a challenge of using linear traits scored at 30 months rather than obtained at 6 months.

4.2 MATERIALS AND METHODS

Description of data

An amount of 7133 records was obtained from data of the stud book of IHDH breed and from the linear type traits information routinely recorded by the National breeder association (ANACAITPR, www.anacaitpr.it). The records prior to 1992, as well as without father and mother, farm, age of birth of foal and mother were eliminated.

The dataset suitable for analyses included single records of 6691 subjects (5835 females, 856 males, including 3585 dams with scored foals and 861 sires with scored foals), aged about 30 months (the average age was 2.83 years), and 11012 individuals in pedigree. Animals were scored linearly for 14 traits with a 9 point scale system (from 1 unfair, to 5 excellent, including half points) by 33 classifiers in 19 subsequent years of evaluation (i.e. from 1992 to 2011). The final conformation overall score, measured only in the adult age, was also considered.

The traits were classified in three class as follows: i) Traits of general aspect: head size (HS), temperament (Te), frame size (FS), fleshiness (Fl), bone incidence (BI); ii) Traits of the trunk: thorax depth (TD), fore diameters (FD), rear diameters (RD), upper line length (UL), upper line direction (UD); iii) Traits of the limbs: legs side view (LS), fore feet (FF), rear feet (RF), hind legs back view (HL). In IHDH breeding management, animals with a light head (HS) are preferred for selection, as well as with a great reactivity to environmental stimuli and a regular pace (Te). High scores of FS are preferred for selection. This trait considers the correct trunk-stature proportion, and it is calculated using a weighted sum including FD, RD, TD and the height. A great development of muscles masses of croup, thigh, buttock, loins and withers (all considered for scoring FI), a fine boned frame (BI), a depth thorax (TD), and large chest (FD) and croup (RD) are also desirable. Intermediate optima are related to traits scoring the correctness of conformation, that are UL, UD, LS, FF, RF and HL. The last 3 traits are scored only at 30 months. The TMI accounts for HS (25% of economic weight), Te (15%), FI (25%), FD (15%) and RD (20%), whose are the most important traits for the selection goal defined by the National Breeders Association (Mantovani et al., 2010b).

The study also considered the overall score (OS) of conformation, also scored only on adult animals, and assigning a final morphological judgement from Fair to Excellent (Folla et al., 2013).

Estimates of (co)variance components, correlations and genetic trend

Data were analysed accordingly to the sex of foal, and the age of foal at scoring (in month). The combination of classifier by year was considered in the model of analysis to take into account the effect of each classifier for every year of evaluation, as in Samoré et al. (1997) for Italian Haflinger.

A variable called “stud group” was formed to consider the effect of the small studs, i.e., with less than 2 scored foals born from different stallions within a year of evaluation. This was carried out on the basis of geographical position and management (stable, pasture and stable or outdoor), the farm’s production goal (production of foals for heavy draught or fattening), the general prophylaxis on foals (vaccination or not) and the mean value mares’ body condition registered at foals‘ evaluation. In this way, groups have been created for neighbouring studs with similar nutrition and management (Folla and Mantovani, 2013). Therefore the effect included in the model was a combination between the group of herd (stud group), the year of birth of foal and the classifier.

A preliminary ANOVA (PROC GLM; SAS Inst. Inc., Cary, NC) was carried out on the non-genetic effects to take into account in the genetic model. The combination of stud group-classifier-year of birth (SYC, 1663 levels), the sex of animals (2 levels), the age at scoring (5 classes, i.e., ≤27, 28, 29-32, 33-47, and ≥48 months of age) were included as effects. Results from preliminary analyses suggested the possible use of multivariate analysis using the same model for all traits.

Variances of each trait and (CO)variance components for all the 14 linear type traits and the overall scor, and for each pairwise combination between traits were estimates via Average Information REML method (AIREML; Johnson and Thompson, 1995) using single and bi-trait animal models and running the AIREMLF90 program (Misztal, 2008). Preliminary AIREML analyses included the SYC effect either as fixed or random. The final model adopted for analysis was written as follows:

$$\mathbf{y=X\beta+Zu+e},$$

where: **y** was the vector of observations for one of the 14 traits recorded on a single animal; **β** was the vector of the same fixed effects of the ANOVA; **u** was the vector of the the random additive genetic effect (11,012 levels, as p animals in pedigree); **e** was the vector of the random residual terms; **X** and **Z** were the incidence matrices assigning observations to the related effects.

The assumptions about the structure of (co)variances for each bi-trait analysis were written as:

$$\mathbf{Var}\begin{vmatrix}\mathbf{u}\\\mathbf{e}\end{vmatrix}=\begin{vmatrix}\mathbf{G}\otimes\mathbf{A} & \mathbf{0}\\\mathbf{0} & \mathbf{R}\otimes\mathbf{I}\end{vmatrix};\ \mathbf{G}=\begin{vmatrix}\sigma^2_{a1} & \sigma_{a12}\\\sigma_{a12} & \sigma^2_{a2}\end{vmatrix};\ \mathbf{R}=\begin{vmatrix}\sigma^2_{e1} & \sigma_{e12}\\\sigma_{e12} & \sigma^2_{e2}\end{vmatrix},$$

where **G** was an additive genetic covariance matrix of order 2x2, **A** the additive genetic relationships matrix for p animals, **R** a residual covariance matrix of order 2x2, **I** an identity matrix, $\otimes$ is the Kronecker product operator, σ_{a1}^2, σ_{a2}^2, σ_{a12} are respectively the additive genetic variances for the two traits and their covariance, and σ_{e1}^2, σ_{e2}^2, σ_{e12} the residual (co)variances for the traits.

The standard errors of the heritability (SE_{h^2}) were computed applying the following formula of Lynch and Walsh (1998):

$$SE_{h^2}=h^2\times\left(\frac{Var(\sigma^2_a)}{(\sigma^2_a)^2}+\frac{Var(\sigma^2_p)}{(\sigma^2_p)^2}-\frac{2Cov(\sigma^2_a,\sigma^2_p)}{\sigma^2_a\sigma^2_p}\right)^{0.5},$$

where h^2 is heritability of a given trait, σ_a^2 and σ_p^2 are the additive genetic and phenotypic variances of the trait, $Var(\sigma_a^2)$, $Var(\sigma_p^2)$ are their respective predicted error variances, and $Cov(\sigma_a^2,\sigma_p^2)$ is the predicted error (co)variance. Furthermore, standard errors of genetic and phenotypic correlations (SE_r) were computed as follows (Lynch and Walsh, 1998):

$$SE_r = r \times \left(\frac{Var(\sigma_1^2)}{4(\sigma_1^2)^2} + \frac{Var(\sigma_2^2)}{4(\sigma_2^2)^2} + \frac{Var(\sigma_{12})}{\sigma_{12}^2} + \frac{2Cov(\sigma_1^2,\sigma_2^2)}{4\sigma_1^2\sigma_2^2} - \frac{2Cov(\sigma_1^2,\sigma_{12})}{2\sigma_1^2\sigma_{12}} - \frac{2Cov(\sigma_{12},\sigma_2^2)}{2\sigma_{12}\sigma_2^2} \right)^{0.5}$$

where r is the correlation between the two traits (genetic, r_g, or phenotypic, r_p), and the other terms are the (co)variances of the traits (1 and 2), and their predicted error (co)variances.

Significance of phenotypic and genetic correlations was tested following Kohn and Atchley (1988).

The genetic trends for all traits of the study were traced from the average breeding values (EBVs) of individuals born in the same year found running a BLUP analysis after AIREML estimations (BLUPF90 program; Misztal et al., 2008), and considering a temporal window of 20 years, from 1992 to 2011. EBVs were standardized to have mean value of 100 and standard deviation of 10.

4.3 RESULTS

Description of data

Means and standard deviations of traits, as well as minimum and maximum values of their scores are reported in Table 2. The means ranged from 2.05 (overall score) to 3.56 (thorax depth), and standard deviation were in the range from 0.33 (hind legs back view) to 0.80 (overall score). Most traits had a mean close to 3 (i.e., the mean point of the linear scale). The traits showing the greatest average evaluations were thorax depth (3.56), rear diameters (3.38), upper line direction (3.30), temperament (3.29) and fleshiness (3.29). Conversely, the traits exhibiting the lowest evaluations were fore feet (2.05), legs side view (2.55), bone incidence (2.86) and upper line direction (2.83). Standard deviations of traits ranged from 0.33 (hind legs back view) to 0.79 (overall score).

In table 2 are reported also skewness and kurtosis values. Skewness ranged from -1.80 (upper line direction) to 0.65 (upper line lenght). A strong right asymmetry of the distribution (negative values of skew) was also found for bone incidence and hind legs back view, whereas a moderate left asymmetry (positive values of skew) was reported for fore feet and overall score. Fleshiness, rear diameters and rear feet showed range values near zero. Kurtosis values ranged from -1.06 (legs side view) to 4.40 (hind legs back view). Most of traits showed a moderately broad distribution (negative values of kurtosis), whereas bone incidence and the traits with intermediate optima excluding LS reported a narrow distribution.

Estimates of (co)variance components

The results of preliminary ANOVA are reported in Table 3. The SYC effect was significant for all traits considered ($P<0.001$), as well the sex, except for temperament, thorax depth, rear diameters, legs side view, hind legs back view and overall score. The age at evaluation was significant only for temperament, frame size, fleshiness, thorax depth, fore and rear diameters, legs side view and overall score. Residual variance, expressed as root mean square error, ranged from 0.08 (hind legs back view) to 0.43 (overall score).

Estimated variances, heritability and standard error are presented in Table 4, also reporting the Akaike Information Criteria (AIC; Akaike, 1974) as model fitting statistics obtained running the AIREML analyses. The AIC was used in preliminary analyses to evaluate whether considering the SYC either as fixed or random effect. The fixed SYC allowed to obtain lower AIC values for all traits (data not shown) and was retained for the study, consistently with a previous work (Folla et al., Chapter 3).

The genetic variances of traits under study (Table 4) ranged from $\sigma^2_a=0.28$ (upper line length) to $\sigma^2_a=13.98$ (frame size), and their standard errors from 0.19 (legs back view) to 1.47 (overall score). The traits showing the greatest genetic variance were frame size ($\sigma^2_a=13.98$), overall score ($\sigma^2_a=13.47$) and head size ($\sigma^2_a=10.09$), while the traits with the lowest values were upper line length ($\sigma^2_a=0.28$), rear feet ($\sigma^2_a=0.40$), and legs back view ($\sigma^2_a=0.54$). Residual variances were higher than genetic ones and ranged from $\sigma^2_e=7.98$ (legs back view) to $\sigma^2_e=30.22$ (overall score). Traits with the higher values were overall score ($\sigma^2_e=30.22$), head size ($\sigma^2_e=22.20$) and frame size ($\sigma^2_e=20.92$), whereas lower values were found for legs back view ($\sigma^2_e=7.98$) and upper line length ($\sigma^2_e=8.41$).

Heritabilities obtained for linear type traits were low or moderate depending on traits, and ranged from $h^2=0.03$ to $h^2=0.40$. Analysing the results, the most heritable traits were frame size ($h^2=0.40$), head size ($h^2=0.31$), fore diameters ($h^2=0.31$) and overall score ($h^2=0.31$) while the lowest values were obtained for the correctness traits of upper line length ($h^2=0.03$), rear feet ($h^2=0.03$), legs back view ($h^2=0.06$) and fore feet ($h^2=0.08$). Temperament, fleshiness, thorax depth and rear diameters had moderate heritabilities (ranging from h^2=0.21 to h^2=0.27). Standard errors of heritability were low and ranged from SE_{h^2}=0.017 (rear feet) to SE_{h^2}=0.033(frame size).

Genetic and phenotypic correlations

Estimates of genetic and phenotypic correlations between pairs of traits are reported in Table 5. The most negative genetic correlation were found between upper line direction and rear feet (r_g=-0.99), and between bone incidence with both temperament (r_g=-0.74) and head size (r_g=-0.64). Bone incidence was also negatively correlated with legs side view and overall score, but the r_g not significantly differed from zero. Legs side view was negative correlated with most traits (r_g ranged from -0.39 to 0.25, but some r_g did not differed from zero). The greatest genetic correlations were found between fleshiness and both fore diameters (r_g=0.74) and rear diameters (r_g=0.91), and between rear diameters with fore diameters (r_g=0.85). Also the thorax depth showed a good correlation with fleshiness (r_g=0.55), fore and rear diameters (r_g=0.56, r_g=0.74). The trait was also highly correlated with frame size too (r_g=0.71), the latter highly correlated also with rear diameters (r_g=0.73) and moderately with fleshiness (r_g=0.45), fore diameters (r_g=0.52) and upper line length (r_g=0.41) and direction (r_g=0.38). Fleshiness had a low correlation with bone incidence (r_g=0.07) that is not different from zero. Moderate and significant positive genetic correlations of bone incidence regarded frame size (r_g=0.22) and fore feet (r_g=0.36), that were also positively related with fleshiness (r_g=0.33) and fore diameters (r_g=0.35). Head size has a great positive genetic correlation with temperament (r_g=0.67), and a moderate but significant r_g with fore diameters (r_g=0.18). Temperament was moderately correlated also with frame size (r_g=0.24) and legs back view (r_g=0.25).

The overall score was significantly positively correlated with most of traits: head size (r_g=0.45), temperament (r_g=0.47). frame size (r_g=0.85), muscularity (r_g=0.61), thorax depth (r_g=0.72), fore and rear diameters (r_g=0.70, r_g=0.77). Negative but not different from zero r_g were found only with fleshiness, rear feet and legs back view.

The approximate genetic standard errors (Table 5) were medium-low, ranging from SE_{rg}=0.001 to SE_{rg}=0.327. Genetic correlations with great SE_{rg} were generally not different from zero.

Generally phenotypic correlations had the same sign but lower values than the respective genetic correlations (Table 5). Different signs were found when the correlations were not different from zero. The trait that showed the greatest correlations with the others was the overall score with frame size (r_p=0.58), rear (r_p=0.54) and fore diameters (r_p=0.52). Moreover also rear diameters exhibited a positive and medium-high correlation with fleshiness (r_p=0.51), like also fore diameters (r_p=0.50). Fleshiness showed positive correlations with fore diameters (r_p=0.47) and rear diameters (r_p=0.51). Another positive phenotypic correlations was fore between diameters with frame size (r_p =0.47). The legs side view was negatively correlated with most traits (r_p ranged from -0.10 to 0.11). The lowest phenotypic correlations were found between head size and bone incidence (r_p=-0.29) and temperament with bone incidence (r_p=-0.18).

The standard errors of phenotypic correlations were lower than genetic ones and ranged from SE_{rp}=0.011 to SE_{rp}=0.016.

Genetic trends of traits

The temporal variation of traits in the twenty years window considered are reported in Figure 1. Traits recorded in young age were used for the genetic improvement, then looking at the trend of traits recorded at adult age it is possible see that results of selection. Figure 1a shows the general traits. The traits involved in TMI (head size, temperament and muscularity) had a positive trend. Frame size, that includes traits under selection in its computation (FD and RD), followed the trend of the other traits. Bone incidence, not included in the TMI, showed a negative trend.

Figure 1b reported the trunk traits, among which the traits with the greatest positive trend were fore and rear diameters, both traits involved in the TMI, also thorax depth showed a positive trend despite not included in TMI. Upper line length and direction, not selected and with intermediate optima, had a positive but lower trend.

Figure 1c reported the limb traits, neither of these included in the TMI, and all of them with intermediate optima. Fore feet and hind legs back view showed a low genetic increase over years, while rear feet and legs side view a low negative trend. The overall score, also reported in Figure 1c, showed a positive variation over time, meaning that the selection carried out in young foals is effective also for adult animals.

4.4 DISCUSSION

A first estimation of genetic parameters for type traits recorded in adult age in Italian Heavy Draught breed horse has been provided in the present study. Genetic evaluation for IHDH, based on linear scoring, offers new insights in the framework of the genetic studies about conformation traits in horse breeds, as IHDH is currently the only horse breed in which genetic parameters have been estimated for traits expressly scored for meat production. Furthermore, the evaluations considered in this study have been realized at the age in which in many horse breeds individuals are scored to be admitted at stud book. An overview of the age at scoring in different horse breeds is reported in Table 1. Horses are usually valued at the age of about 3-4 years or more, as reported for Dutch Warmblood horse (Koenen et al., 1995) and for Andalusian (Molina et al., 1999). An age at evaluation of at least 30 months has regarded also draught breeds such as Italian Haflinger (Samoré et al., 1997), Noriker (Druml et al., 2008), and Posavje (Simčič et al., 2012), despite draught horses are generally considered early-maturing (Druml et al., 2008). The IHDH is currently valued at 2-7 months of age to ease a rapid genetic improvement (Mantovani et al., 2005), but some differences in the heritable components of traits may be disclosed in 6 months (Folla et al., Chapter 3) and in 30-months aged animals: in adults frame size h^2 is 14 percentage points greater, maybe due to a greater genetic variability in growth, whereas fleshiness h^2 decreases of 10 percentage points, maybe because after selection adult animals could have more similar genetic values than foals.

Table 1 also provides an overview of the evaluation systems for conformation that over years have been applied in horse breeds. Evaluations of conformation traits derived from different breeds and evaluation system are difficult to compare, because of noteworthy differences in breeding goals and in the evaluation (Druml et al., 2008). The direct measurement of body regions has been widely accounted in horse literature, such in Andalusian horse (Molina et al., 1999), Lipizzan (Zechner et al., 2001), and Noriker (Druml et al., 2008). Morphometric measurements have received recent improvements by the software for image analysis of individual body pictures, as for Spanish Arab Horse (Cervantes et al., 2009). Since the first evaluation system proposed in 1989 for Dutch Warmblood (Koenen et al., 1995), many studies have been based on linear scoring system, where a number of traits are individually scored along a biological scale to evaluate body regions (e.g., in

Shetland pony, Van Bergen and Van Arendonk, 1993; in Italian Haflinger, Samoré et al., 1997). In some other cases, traits are subjectively scored (e.g., in Trakehner horse, Preisinger et al., 1991), or are a combination of biological and subjective scoring (e.g., in Noriker horse, Druml et al., 2008). Linear traits, as well as morphometric measurements, have been widely introduced over years because they provide more objective methodologies assessing conformation than traditional subjectively scoring, typically more influenced by environmental factors (Saastamoinen and Barrey, 2000; Cervantes et al., 2009). Linear scoring system also allows to score a large number of conformation individually rather than in combination (Vostrý et al., 2011). Scoring traits individually may allow to more easily reveal the differences in conformation between animals, as compared to situations in which different traits are combined (Druml et al., 2008)..

Linear type evaluation in IHDH involves a majority of traits that is individually scored plus a pair of traits that are a combination of others, i.e. frame size and fleshiness, and a final morphological overall score that is subjectively scored. Genetic parameters estimated in the present study concern traits that have been also valued in a number of horse populations, while about the traits related to muscular development (summarised in fleshiness in IHDH), the estimates obtained in this study are difficult to compare within other horse breeds, due to the lack of similar studies. Comparison can be made with cattle hypertrophic breeds. Looking at literature on this topic, heritability estimates reported by Albera et al. (2001) on fleshiness in Piedmontese young bulls ranged from h^2=0.26 to h^2=0.55. A further analysis on Piedmontese cows (Mantovani et al., 2010a) reported an average heritability of h^2=0.12 for linear type traits scoring muscles in withers, shoulder, loin and thigh. Again, Hansen et al. (1998), estimated heritabilities between h^2=0.36 and h^2=0.41 for traits correlated with thigh muscularity in Belgian Blue cows, while Gutiérrez et al (2002) reported a value of h^2=0.22 for trait correlated with muscularity (thigh development) in Spanish Asturiana beef cattle. Then, Veselá et al (2005) founded values ranging from h^2=0.25 to h^2=0.34 for shoulder, back, and rump muscling scores in by Czech Beef Cattle. Not included in selection index but also important for meat evaluation since it indicates the further incidence of bones in the animal carcass, bone incidence is another trait not scored in horses excluding IHDH, but recorded in beef cattle. An estimation of the heritability of the trait (here called bone thinness) has been reported e.g. for Piedmontese cattle (Mantovani et al., 2010a), and was close (h^2=0.12) to the one of this study.

Other traits of interest for meat purpose, i.e., , fore diameters and rear diameters, have been valued also in other horse breeds than IHDH because important for the heavy draught. In this study the heritability of fore diameters was 0.31. Different results are reported by other Authors that linearly scored the same body part: Druml et al. (2008) found a h^2=0.16 for fore quarter in Noriker horse, and by Molina et al. (1999) and Miserani et al. (2002), that estimated heritabilities of 0.40 and of 0.51, respectively, for chest width in Andalusian and Panteiro horses. One of the first estimation on linear type traits, performed by Van Bergen and Van Arendonk (1993) on Shetland pony, reported a h^2=0.18, whereas the recent work of Vicente et al. (2014) on Lusitano horse found a lower value of h^2=0.12 on chest and thorax trait. Finally Bakhtiari et al (2009) found a value of 0.22 for the morphometric measurement of chest width. Some works (Molina et al., 1999, Vicente et al., 2014) have included in the same evaluation both chest and thorax, that have been separately scored in IHDH, in which they reported different heritabilities (h^2=0.21 for thorax depth).

The rear diameters heritability (h^2=0.27) in the IHDH is higher than those found in Italian Haflinger by Samoré et al (1997), that presented a value of h^2=0.11 for croup width, while similar values were found by Koenen et al. (1995) in Dutch Warmblood (h^2=0.28), Druml et al (2008) in Noriker (h^2=0.20), and Fioretti et al. (2005) in Bardigiano (h^2=0.25). A greater value of h^2=0.59 was found in Panteiro horse by Miserani et al. (2002) for croup height, whereas Vicente et al (2014) found a lower value of h^2=0.15 for croup in Lusitano horse.

Summing the evaluations of diameters, thorax and of the height, the combined trait of frame size reported the greatest h^2 in IHDH (h^2=0.40). An overall evaluation of the frame has been found also in few other horse breeds, as in Hanoverian Warmblood (h^2=0.20; Schröder et al. 2010).

About the other traits under selection in IHDH, head size (h^2=0.31) is widely evaluated in horse breeds regardless the breeding purpose. Lower heritabilities (h^2=0.11) were found by Druml et al. (2008; h^2=0.11) for the Noriker horse that is light draft breed, and by Samoré et al (1997), that averaging the values of head volume and expression found a value of h^2=0.24 in Italian Haflinger. Again, Molina et al.

(1999), reported a value of $h^2=0.23$ for head and neck in Andalusian horse, and a value of $h^2=0.21$ for the same trait was estimated by Koenen et al. (1995) in Dutch Warmblood. A similar value ($h^2=0.18$) for head and neck was found by Vicente et al (2014) in Lusitano horse. Contrarily, in the Bardigiano horse Fioretti et al (2005), reported three h^2 values of head (volume: $h^2=0.47$, profile: $h^2=0.33$ and expression: $h^2=0.39$). In the Pantaneiro horse, that is a dual purpose breed, like a IHDH, used for beef and draft, Miserani et al (2002) found different heritabilities for head length ($h^2=0.55$) and head width ($h^2=0.27$). Another high heritability was estimated by Schröder et al. (2010), that presented a value of $h^2=0.47$ for head. Finally, Bakhtiari et al (2009) reported a value of $h^2=0.39$ for head length in the Iranian horse breed, but the estimate was a morphometric measure and not a linear evaluation as in the other breeds. The reason of the great differences among h^2 estimates are probably due to the breed and the purpose of selection, and to the method of evaluations (e.g. in the Haflinger, Andalusian and Noriker use a scale from 1 to 10, while Iranian horse use only body measurements).

The last trait included in the selection index, the temperament, is not a type traits, but it is often scored in horse breeds because it concurs to the general framework of individual body appearance. In IHDH the trait evaluated both the docility and the regularity of the movement, often separately considered in other horse breeds. The heritability ($h^2=0.21$) for temperament is higher than those found by Samoré et al. (1997) for temperament ($h^2=0.06$), but close to the h^2 value for the gait ($h^2=0.19$). Similarly, Molina et al. (1999) reported an heritability of 0.08 for temperament, but of 0.15 for movement, and a similar value to in IHDH ($h^2=0.20$) was found by Druml et al. (2008) for movement too.

The heritabilities of the traits scoring the correctness of body and legs are low (values ranged from 0.06 to 0.12), and that's probably because these traits are related to a proper conformation of the animal. The heritabilities for leg stances linearly scored in some horse breeds, summarized by Saastamoinen and Barrey (2000), ranged from $h^2=0.07$ to $h^2=0.21$ in Shetland pony (Van Bergen and Van Arendonk., 1993), from $h^2=0.14$ to $h^2=0.23$ in Dutch Warmblood horse (Koenen et al., 1995), and from $h^2=0.10$ to $h^2=0.17$ in Italian Haflinger (Samoré et al., 1997). The recent estimation (Vicente et al., 2014) for Lusitano breed also reported a low heritability value for legs ($h^2=0.07$). An estimation of h^2 for the upper line length was provided by Vostrý et al.

(2011) for the Old Kladruber horse, and assumed an higher value (h^2=0.28) than in IHDH.

A final morphological overall score, reporting in IHDH an heritability of 0.40, was also evaluated in other horse breeds to summarize the evaluation of conformation: type in Noriker draught horse showed a h^2=0.37 close to the IHDH one, whereas a lower estimate (h^2=0.14) was found in the riding breed Lusitano horse (Vicente et al., 2014). Different aspects are likely to be valued for providing an overall morphological judgement in breeds with different purposes, and the heritabilities estimated, as well as the genetic correlations with the other traits scored reflect the selection purposes of the breeds (Saastamoinen and Barrey, 2000). The only positive and different from zero genetic correlations of the overall score with the other traits in IHDH where those with the traits included in TMI or positively correlated at genetic level with muscular development, and this indicates the importance of such traits in the final appreciation of candidate mares and stallions. High genetic correlations between the overall score and the other traits of interest were also found in Noriker horse (Druml et al., 2008).

The genetic correlations among the linear type traits scored in IHDH reflect on some extent the breeding purposes of the breed. The high and negative genetic correlation between head and bone incidence (r_g=-0.64) is consistent with the fact that in IHDH selection they are preferred subjects with not much voluminous head (ANACAITPR, 2010). This is important because bone incidence directly correlates with the yield at abbatage, lower if bone incidence in carcass is greater. High and positive genetic correlations between head and temperament, underlining a general good appearance of the animals in aspect and movement, desirable in IHDH selection (Mantovani et al., 2010b), has been also found in other breeds e.g. in Noriker draught horse (Druml et al., 2008), reporting a correlation between head and movement of 0.66. The head size is positive correlated with temperament because neck and head are important for the balance of animal and subsequently they influence the movement. In IHDH selection they are preferred horses with head not too voluminous and neck not too short, but with good development of muscular mass (ANACAITPR, 2010).

Genetic correlations between head and traits related to muscularity excluding fore diameters did not differed from zero. Similarly Vicente et al (2014), found a low genetic correlations between head-neck and chest-thorax (r_g=0.081), but a greater r_g=0.24 between head-neck and croup. Molina et al (1999) reported a genetic correlation between head-neck, and chest-thorax of r_g=0.22, and the same value for the genetic correlation between croup-tail and head-neck, while Druml et al. (2008), that valuated neck and head separately, reported a genetic correlation between morphological measures of head and chest circumference of r_g=0.74, and between head and rear quarterof r_g=0.58. Bakhtiari et al (2009) found a correlation of r_g=0.52 between head length and chest width morphometric measures. Differences in correlations may be due to the evaluation system (morphometric measurements or linear scoring) and to the different ways to score the head: in IHDH the highest scores are provided to animals with a smaller head, whereas in other breeds different aspects as the shape of the head are valued (e.g., in Lusitano; Vicente et al., 2014).

The high and positive genetic correlations of frame size with thorax depth and rear and fore diameters are because in the evaluation of frame size are also included the two traits, whereas the high and positive genetic correlations of fleshiness with fore and rear diameters and thorax depth reflect the fact that wide diameters offer more space for muscle masses, and the thorax develops consistently with diameters. Fleshiness had a low and not different from zero genetic correlation with bone incidence, as expected since bone incidence is measured in relation to the muscle development (ANACAITPR, 2010).

Briefly looking at horse literature, Vicente et al (2014) found an high value of r_g between chest-thorax and croup (r_g =0.52), and a value close to one was found between chest and thorax in Panteiro horse (Miserani et al., 2002). Kashiwamura et al (2001), found an average genetic correlation between chest width and croup width of r_g=0.37 in Banei Draught Racehorse.

The genetic correlations in beef cattle among traits correlated with muscularity and considered in this study are positive and high, e.g. in the Asturiana breed Gutiérrez et al. (2002) found values of r_g ranging from r_g=0.74 to r_g=0.88 (fore quarters, rump, thigh development), while Norris et al (2008) in the Charolaise breed reported values ranging from r_g=0.48 to r_g=0.95 (hind round, hip width, rump flat, form and length).

The genetic correlations among traits of conformation correctness suggest strong relationships between the defects occurred in different body parts, in particular between the upper line direction and in the rear feet, and between fore and rear feet.

An example of high genetic correlation (r_g=0.61) between correctness and hind feet has been reported in Noriker horse (Druml et al., 2008).

The genetic trends of traits are consistent with traits heritability, with the genetic correlations among traits, with the selection purposes of the breeds and with the biological meaning of the linear scoring. The trend is constantly positive in traits included within the selection index as well as in traits highly genetically correlated with them. Breeders prefer nevrile horse, indeed temperament showed a slower increase than in other traits due to a lower heritability. Bone incidence, negatively correlated with head size showed a trend close to zero. The slight genetic variation in correctness traits is because traits have intermediate optima, and the best individual breeding values for these traits are the mean ones. The genetic variations over years observed in this study follow the positive genetic trends already provided for temperament and fleshiness scored at 6 months of age (Mantovani et al., 2005), and suggest that the selection carried out in young foals is factual also for the genetic improvement for traits scored at 30 months of age. A genetic evaluation based on traits scored at adult age may therefore be factual as well as the current one carried out on young foals. A proper knowledge of genetic correlation among traits at 6 and 30 months, currently under study (Folla et al., Chapter 3), will be useful for the future to substitute the evaluation at 6 months with 30 months. That will allow to reduce of animal evaluated and consistently the costs.

4.5 CONCLUSIONS

To conclude, it is possible to observe that genetic parameters of traits recorded at 30 months of age are consistent with estimates obtained in traits recorded in young foals (Folla et al. Chapter 3) and currently used for genetic improvement. Genetic evaluation in Italian Heavy Draught Horse Breed is based on a linear type evaluation of 11 traits scored at 6 months of age, 5 of them included in the selection index of the breed. At adult age the evaluation is performed again on the same traits and 3 further traits of legs correctness, as well as an overall score for morphology, are included.

The results about morphological evaluation indicate that the goal of selection is to obtain an animal with a good muscular mass but elegant and brilliant in the movements. The traits involved in Total Merit Index include indeed head size, temperament, fleshiness, fore diameters, rear diameters, that have a medium high heritability (ranging from $h^2=0.21$ to $h^2=0.31$). The traits involved in the muscular development, that are fleshiness and fore and rear diameters, had moderate high heritability estimates,that indicates that selection can be used for these traits and a suitable response will be found. These traits are all positively correlated, and the genetics correlations were very high, ranging from $r_g=0.74$ to $r_g=0.91$, while phenotypic correlations were lower, ranging from $r_g=0.47$ to $r_g=0.51$. Most of genetic correlations between traits included in the selection index are moderate-high, meaning that selection for one of these traits should result in an increase in the other traits of interest. Positive genetic trends have been observed for traits of selection interest despite breeding values are estimated on traits scored at young age. Evaluations at adult age are however mandatory for the final admission at stud book of candidate stallions and mares. This study has shown that the use of traits scored at adult age instead that in foals is feasible in IHDH selection.

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***Table 1**. Overview of morphological evaluation in some horse breeds.*

Breed	Aptitude	Evaluation	Age at evaluation	Type of traits	Reference
Czech-Moravian Belgian	Draft	Linear type/body measurements	-	Morphological	L. Vostrý 2009
Noriker	Draft	Body measurements	≥3 years	Conformation	T. Druml 2008
Old Kladrub	Sport	Linear type/body measurements	-	Conformation	V. Jakubec 2007
Posavje	Draft	linear Type/measurements	30-60 months	Conformation	M. Simčič 2012
Banei	Draft	Body measurements	≥2 years	Conformation	F. Kashiwamura 2001
Wielkopolski	Sport	Body measurements	-	Conformation	M. Kaproń 2013
Lipizzan	Sport	Body measurements	≥4 years	Morphological	P. Zechner 2001
Andalusian	Sport	Body measurements	≥4 years	Morphofunctional	A. Molina 1999
Dutch Warmblood Riding	Sport	Linear type traits	3-7 years	Conformation	V. Jakubec 2007
Haflinger	Sport	Linear type traits	2 years	Conformation	A.B. Samorè 1997
Bardigiano	Saddle	Linear type traits	-	Morphological	M. Fioretti 2009
Danish Warmblood	Sport	Linear type traits	Young age	Conformation	L. Jönsson 2014

***Table 2**. Descriptive statistics of the 15 linear traits scored in 6691 IHDH horses.*

	Descriptor range			Descriptor	
Trait		Skewness	Kurtosis	Minimun	Maximum
Head size (HS)	3.04±0.64	0.05	-0.14	Heavy	Light
Temperament (Te)	3.29±0.54	0.17	0.45	Lymphatic	Nevrile
Frame size (FS)	3.20±0.71	0.07	-0.25	Little	Large
Fleshiness (Fl)	3.28±0.54	-0.03	0.09	Poor	Excellent
Bone incidence (BI)	2.88±0.39	-0.76	2.53	Fine boned	Heavy boned
Thorax depht (TD)	3.55±0.53	-0.19	-0.38	Little	Large
Fore diameters (FD)	2.93±0.65	0.17	-0.25	Narrow	Wide
Rear diameters (RD)	3.37±0.56	0.01	-0.28	Narrow	Wide
Upper line lenght (UL)	3.28±0.45	0.65	-0.37	Short	Long
Upper line direction (UD)	2.85±0.36	-1.80	1.97	Kyphotic	Curved
Legs side view (LS)	2.58±0.47	-0.28	-1.06	Sickle	Straight
Fore feet (FF)	3.22±0.51	0.32	0.52	Diverging	Converging
Rear feet (RF)	3.01±0.42	0.03	2.88	Diverging	Converging
Hind legs back view (HL)	2.90±0.33	-1.73	4.40	Diverging	Converging
Overall score (OS)	2.05±0.79	0.28	-0.51	Fair	Excellent

***Table 3**. Results of preliminary ANOVA (GLM procedure; SAS Inst. Inc., Cary, NC) performed on the 15 linear traits considered in the analyses. Sum of squares are reported for each effect included in the model. Residual variance (root mean square error) for each trait is also reported.*

Trait	Effect			Residual variance
	HYC	SEX	AGE	
Head size (HS)	0.649***	6.063***	0.502	0.32
Temperament (Te)	0.493***	0.243	0.864**	0.22
Frame size (FS)	0.893***	29.514***	1.645***	0.34
Fleshiness (Fl)	0.486***	9.279***	1.571***	0.21
Bone incidence (BI)	0.232***	2.088***	0.032	0.13
Thorax depht (TD)	0.513***	0.077	0.841**	0.20
Fore diameters (FD)	0.758***	25.758***	2.255***	0.28
Rear diameters (RD)	0.534***	<0.001	2.204***	0.23
Upper line lenght (UL)	0.313***	1.939***	0.135	0.16
Upper line direction (UD)	0.259***	2.424***	0.008	0.09
Legs side view (LS)	0.403***	0.190	0.379*	0.15
Fore feet (FF)	0.341***	3.798***	0.451	0.23
Rear feet (RF)	0.261***	2.321***	0.108	0.14
Hind legs back view (HL)	0.169***	0.002	0.104	0.08
Final score (FS)	1.142***	0.277	5.774***	0.43

(1) HYC is the herd-year-classifier effect (1663 levels); SEX is the sex effect (male or female), AGE is the class of age at the time of linear evaluation (5 levels);
*P < 0.05; **P < 0.01; ***P < 0.001; when absent not significant.

***Table 4**. Genetic (σ_a^2), residual (σ_e^2), phenotypic variance (σ_p^2) and their standard errors (SE), heritability (h^2) and its SE and Akaike Information Criterion (AIC; Akaike, 1974).*

Trait	*σ_a^2 (SE)*	*σ_e^2 (SE)*	*σ_p^2 (SE)*	*h^2 (SE)*	*AIC*
Head size (HS)	10.09 (1.15)	22.20 (0.92)	32.29 (0.72)	0.31 (0.03)	39924
Temperament (Te)	4.75 (0.71)	18.01 (0.64)	22.77 (0.49)	0.21 (0.03)	38316
Frame Size(FS)	13.98 (1.35)	20.92 (1.00)	34.9 (0.81)	0.40 (0.03)	40168
Muscularity (Mu)	5.47 (0.71)	16.21 (0.61)	21.68 (0.47)	0.25 (0.03)	38011
Bone Incidence (BI)	1.68 (0.37)	11.31 (0.37)	12.99 (0.27	0.13 (0.03)	35591
Thorax Depth (TD)	4.31 (0.64)	16.00 (0.58)	20.31 (0.43)	0.21 (0.03)	37738
Fore Diameters (FD)	8.95 (1.00)	19.57 (0.81)	28.52 (0.63)	0.31 (0.03)	39298
Rear Diameters (RD)	6.26 (0.79)	17.16 (0.67)	23.42 (0.51)	0.27 (0.03)	38377
Upper Line Length (UL)	1.58 (0.40)	14.79 (0.44)	16.37 (0.34)	0.10 (0.02)	36786
Upper Line Length (UD)	0.28 (0.17)	8.41 (0.22)	8.69 (0.17)	0.03 (0.02)	33658
Rear Legs Side View (LS)	1.94 (0.43)	13.81 (0.44)	15.75 (0.33)	0.12 (0.03)	36567
Fore Feet (FF)	1.85 (0.53)	21.3 (0.61)	23.15 (0.47)	0.08 (0.02)	38542
Rear Feet (RF)	0.40 (0.24)	13.98 (0.35)	14.37 (0.29)	0.03 (0.02)	36189
Legs Back View (LB)	0.54 (0.19)	7.98 (0.22)	8.53 (0.17)	0.06 (0.02)	33540
Overall Score (OS)	13.47 (1.47)	30.22 (1.20)	43.69 (0.96)	0.31 (0.03)	41418

***Table 5**. Estimates genetic correlations and standard errors, fixed effect (above the diagonal) and random effect (below the diagonal), of fifteen type traits, body measurements, and linear description. Descriptions of traits are reported in Table 4.*

Traits	HS	Te	FS	Mu	BI	TD	FD	RD	UL	UD	LS	FF	RF	LB	OS
HS		**0.67** **(0.069)**	0.13 (0.074)	0.12 (0.085)	**-0.64** **(0.088)**	0.14 0.092	**0.18** **(0.078)**	0.10 (0.085)	-0.01 (0.124)	-0.10 (0.185)	0.12 (0.111)	-0.15 (0.130)	-0.23 (0.213)	-0.21 (0.142)	**0.45** **(0.069)**
Te	**0.30** **(0.014)**		**0.24** **(0.083)**	0.09 (0.098)	**-0.74** **(0.104)**	0.15 (0.104)	0.13 (0.090)	0.09 (0.097)	0.18 (0.140)	0.05 (0.219)	**0.25** **(0.122)**	-0.19 (0.146)	-0.15 (0.228)	-0.12 (0.167)	**0.47** **(0.076)**
FS	**0.08** **(0.016)**	**0.13** **(0.015)**		**0.45** **(0.069)**	**0.22** **(0.102)**	**0.71** **(0.060)**	**0.52** **(0.061)**	**0.73** **(0.051)**	**0.41** **(0.108)**	**0.38** **(0.188)**	-0.03 (0.106)	0.13 (0.123)	0.03 (0.189)	-0.10 (0.137)	**0.85** **(0.034)**
Mu	**0.11** **(0.015)**	**0.09** **(0.015)**	**0.31** **(0.014)**		0.07 (0.119)	**0.55** **(0.081)**	**0.74** **(0.053)**	**0.91** **(0.039)**	0.09 (0.131)	-0.18 (0.192)	**-0.33** **(0.125)**	**0.33** **(0.127)**	0.09 (0.209)	0.04 (0.154)	**0.61** **(0.061)**
BI	**-0.29** **(0.014)**	**-0.18** **(0.014)**	**0.05** **(0.015)**	**-0.08** **(0.015)**		0.13 (0.126)	0.03 (0.110)	0.15 (0.115)	0.01 (0.168)	0.22 (0.250)	-0.23 (0.149)	**0.36** **(0.170)**	0.40 (0.275)	0.13 (0.199)	-0.13 (0.108)
TD	**0.09** **(0.015)**	**0.05** **(0.015)**	**0.39** **(0.013)**	**0.30** **(0.014)**	0.01 (0.015)		**0.56** **(0.072)**	**0.74** **(0.061)**	0.01 (0.140)	-0.09 (0.212)	**-0.29** **(0.131)**	0.14 (0.146)	0.08 (0.221)	0.13 (0.164)	**0.72** **(0.060)**
FD	**0.14** **(0.015)**	**0.11** **(0.015)**	**0.36** **(0.014)**	**0.47** **(0.012)**	**-0.04** **(0.015)**	**0.36** **(0.013)**		**0.76** **(0.049)**	0.23 (0.123)	0.05 (0.195)	**-0.27** **(0.116)**	**0.35** **(0.122)**	0.26 (0.193)	0.18 (0.145)	**0.70** **(0.049)**
RD	**0.0** **(0.015)**	**0.07** **(0.015)**	**0.44** **(0.012)**	**0.51** **(0.011)**	-0.01 (0.015)	**0.40** **(0.013)**	**0.50** **(0.011)**		**0.34** **(0.126)**	-0.01 (0.201)	**-0.25** **(0.118)**	0.16 (0.134)	0.10 (0.207)	-0.25 (0.149)	**0.77** **(0.047)**
UL	**-0.04** **(0.015)**	**0.03** **(0.015)**	**0.13** **(0.015)**	**-0.05** **(0.015)**	**0.05** **(0.015)**	-0.03 (0.015)	**0.04** **(0.015)**	**0.03** **(0.015)**		0.14 (0.273)	0.04 (0.169)	0.15 (0.193)	-0.17 (0.265)	0.16 (0.209)	0.19 (0.122)
UD	0.01 (0.015)	**0.03** **(0.014)**	**0.06** **(0.015)**	-0.03 (0.015)	**0.05** **(0.014)**	-0.02 (0.014)	**-0.04** **(0.015)**	-0.01 (0.015)	**-0.06** **(0.014)**		0.10 (0.253)	-0.12 (0.285)	**-0.99** **(0.007)**	-0.21 (0.327)	0.23 (0.201)
LS	**0.10** **(0.015)**	**0.11** **(0.015)**	-0.03 (0.015)	**-0.04** **(0.015)**	**-0.10** **(0.015)**	**-0.04** **(0.015)**	-0.01 (0.015)	**-0.06** **(0.015)**	**-0.07** **(0.014)**	0.02 (0.014)		-0.13 (0.175)	-0.24 (0.259)	**0.39** **(0.182)**	0.05 (0.110)
FF	-0.01 (0.015)	-0.03 (0.015)	**0.04** **(0.015)**	**0.08** **(0.015)**	0.01 (0.015)	**0.06** **(0.015)**	**0.12** **(0.015)**	**0.11** **(0.015)**	0.02 (0.014)	-0.01 (0.014)	-0.03 (0.014)		**0.56** **(0.282)**	-0.06 (0.225)	0.10 (0.127)
RF	-0.02 (0.015)	-0.01 (0.014)	0.01 (0.015)	0.02 (0.014)	0.02 (0.014)	0.02 (0.014)	**0.03** **(0.015)**	0.01 (0.014)	0.01 (0.014)	0.01 (0.012)	-0.02 (0.014)	**0.09** **(0.014)**		0.49 (0.309)	-0.11 (0.203)
LB	**0.03** **(0.015)**	0.01 (0.015)	-0.01 (0.015)	0.02 (0.015)	0.01 (0.014)	0.01 (0.015)	**0.04** **(0.015)**	-0.01 (0.015)	-0.01 (0.014)	-0.01 (0.014)	**0.08** **(0.014)**	0.02 (0.014)	**0.10** **(0.014)**		-0.04 (0.143)
OS	**0.31** **(0.014)**	**0.30** **(0.014)**	**0.58** **(0.010)**	**0.46** **(0.012)**	**-0.09** **(0.015)**	**0.41** **(0.013)**	**0.52** **(0.011)**	**0.54** **(0.011)**	0.01 (0.015)	**0.04** **(0.014)**	**0.06** **(0.015)**	**0.06** **(0.015)**	0.01 (0.014)	**0.04** **(0.015)**	

***Figure 1a**. Genetic trends for general linear type traits*

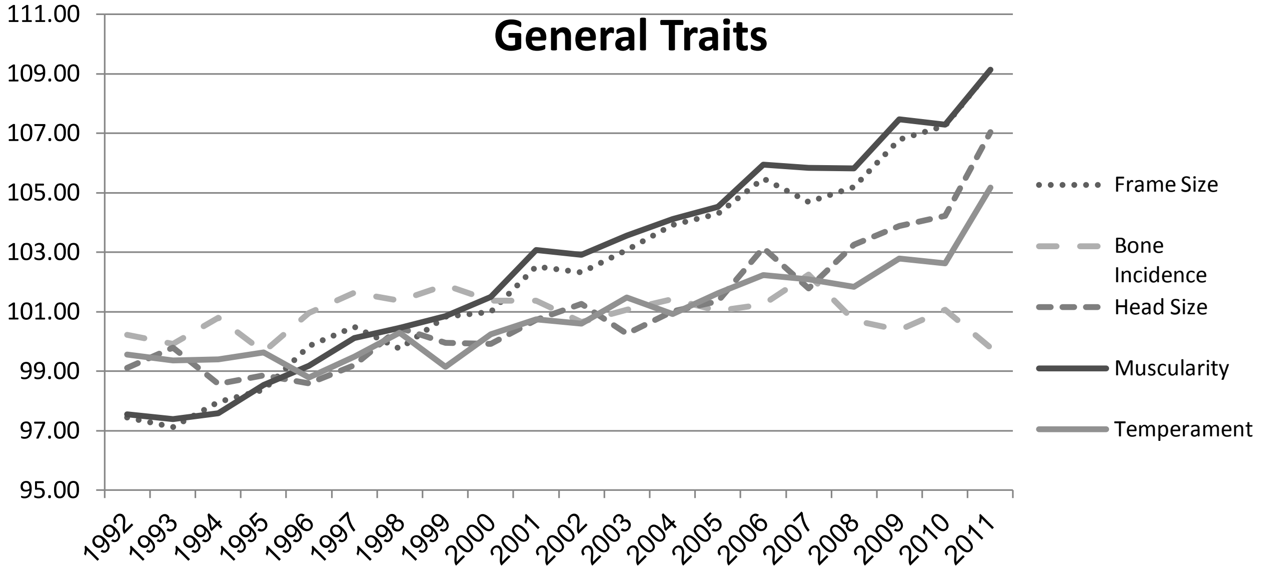


***Figure 1b**. Genetic trends for linear type traits of trunk*

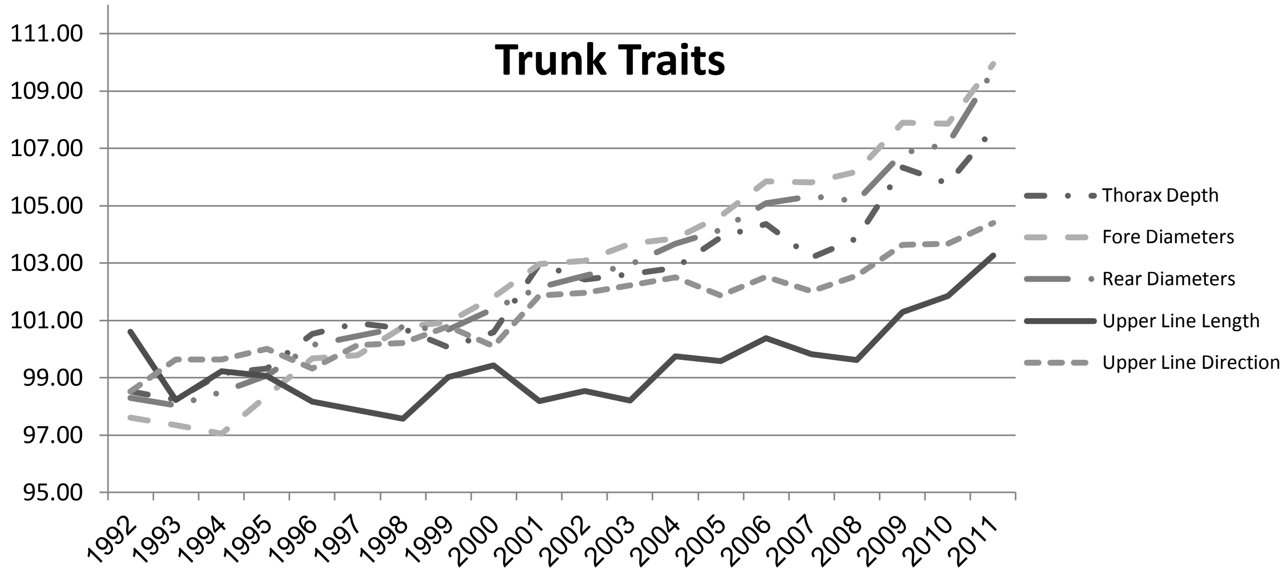
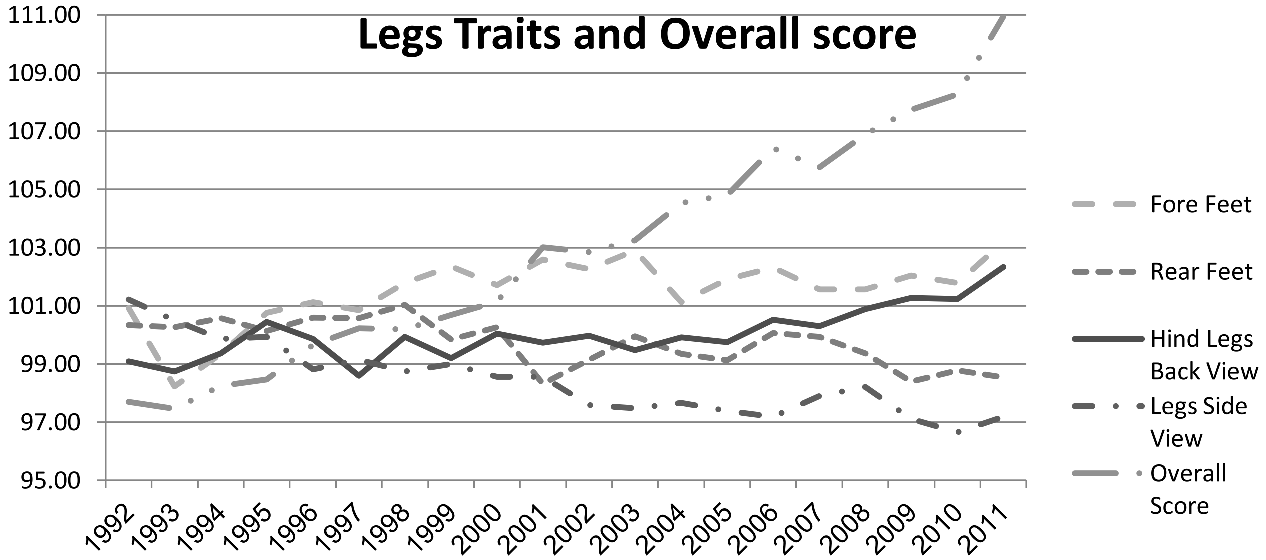


***Figure 1c**. Genetic trends for legs traits and overall score*



CHAPTER 5

GENETIC CORRELATIONS OF TYPE TRAITS SCORED AT 6 AND 30 MONTHS OF AGE IN ITALIAN HEAVY DRAUGHT HORSE AND EFFECTS ON BREEDING PROGRAMME

5.1 INTRODUCTION

Understanding evolutionary changes of traits requires understanding the genetic and environmental components on a trait, as well as the occurring selective pressures (Lynch and Walsh, 1998; Charmantier et al., 2006). The evolutionary response to selection in quantitative traits is proportional to the selection strength and trait's heritability, i.e. the ratio of the additive genetic variance of a trait (its heritable component) and the whole phenotypic variance (Falconer and McKay, 1996). In a multi-trait framework, different selective pressures are contemporarily acting on individual's traits, and the ratio of the additive genetic (co)variances matrix of traits (the **G** matrix) and the matrix of phenotypic (co)variances (**P** matrix) should be considered to predict the multi-trait response to selection (Lande, 1979). Some evidences have shown that **G** matrix can evolve within populations (Steppan et al., 2002) and differ across environments (Hoffmann and Merilä, 1999), selective pressures (Shaw et al., 1995), inbreeding levels (Phillips et al., 2001).

Some changes in the **G** matrix can also occur with respect to the age of individuals (Charmantier et al., 2006). Examples of age-dependence in the expression of additive genetic variance of traits may be found in few studies carried out in laboratory (e.g., in *Drosophila melanogaster*, Hughes et al., 2002), in natural populations (e.g., in mute swan, Charmantier et al., 2006), and in livestock species. The last group mainly includes studies about genetic variation of body weight with age and genetic correlations with other traits, carried out in different species as cattle (e.g., Meyer, 2004), sheeps (e.g., Lewis and Brotherstone, 2002), pigs (e.g., Haraldsen et al., 2009), horses (e.g., Kaps et al., 2010). Further examples of genetic variations in traits recorded at different ages are scarce. Few studies have been performed in horses, in which a number of competition (e.g. trot, dressage, free-jumping) and conformation (e.g. type, correctness of legs) traits have been scored at different ages, or in mares and foals (Teegen et al., 2006; Viklund et al., 2010; Becker et al., 2013; Schöpke et al., 2013). The first issue of these studies is to optimize the selection programs of the breeds, by characterizing the genetic structure of traits, and by investigating how much traits recorded at different ages (e.g., in young foals or in 3-4 years animals) fit with competition or morphological requirements later in life. Heritabilities and genetic correlations between traits scored at different ages have been estimated in these studies, but no inference has been carried out about the predicted response to selection occurring when selective pressures are acting at different ages on individuals.

Some insights on this regards can be offered by the linear type evaluation carried out in the Italian Heavy Draught Horse, or IHDH. The mean goal of selection in the IHDH is meat production and then draft. The selection in this breed is based on evaluation of foals at 6 months and at 30 months (Mantovani et al., 2005). The evaluation consists in the scoring of the same 11 linear type traits at the two ages plus 3 additional traits only in 30-months animals. The same expert classifiers are used to score the animals at both ages using the same evaluation criteria. The evaluation at 6 months is then used in the genetic improvement, while the admission on the studbook of candidate mares and stallions needs the evaluation at 30 months (Mantovani et al., 2005; Folla et al., 2013).

Type classification is a methodology that describes the animal's conformation according to the breed standards about skeletal and muscular development, and it is expected to be useful to evaluate the animal's productive aptitude (Vinson et al., 1982; Lucas et al., 1984). The linear evaluation of type traits is widely applied both in cattle and horse breeding and is based on the use of a discrete scale of points, generally five, nine, or fifty, which extremes correspond to the biological extremes of the traits (Thompson, 1981).

A five-points linear scoring system for genetic improvement of IHDH was introduced in the 1992, and in the 2005 the half points have been introduced (anacaitpr.it). The traits routinely recorded in IHDH belong to 3 main groups of general aspect, trunk, feet and legs (Mantovani et al., 2005), and are expected to be useful for evaluating the animals as meat producers. In this breed, the genetic improvement of meat quality traits may be obtained as a response to selection either in foals or in 30-months-animals traits.

The current choice of the breeders in IHDH considers an aggregated selection index called Total Merit Index or TMI (Mantovani et al., 2005) that includes 5 of the 11 linear type traits scored on young foals and assigns a different weight to each trait considered. The evaluation at 6 months involves about the whole population of young foals, and allows a fast genetic improvement because the animals are evaluated at young age. The subsequent evaluation at 30 months is made only on candidate mares and stallions that have already passed the 6-months-evaluation and is included within the criteria for the admission to the studbook. An overall score (OS) is also recorded at 30 months as linear type traits and used to summarize the physical appearance of the animal (anacaitpr.it). The possibility to use in the TMI the scores on adults instead of the ones on foals is currently discussed in IHDH breed management, especially because less subjects are yearly valued among 30-months animals (about 450 animals/year vs. 800 foals/year; Folla et al., 2013).

The knowledge of the genetic correlations among traits in young and adult age is important because it allows to evaluate the effects of substituting in genetic improvement the evaluations at young age with that ones at adult age, that would allow a cost saving. First investigations on genetic parameters in traits scored either at 6 or at 30 months in IHDH have been already separately performed (Folla et al., Chapter 3; Folla et al., Chapter 4), but no relationships between traits scored at different ages have been investigated yet.

The present study aims to estimate the genetic correlations between the same traits recorded at 6 and 30 months in IHDH, and to evaluate the possible differences in genetic improvement that may be due to the use of the linear type traits scored at young or adult ages by measuring the genetic correlations of traits with the OS, and by comparing the different response to selection when traits of foals or of mares are used.

5.2 MATERIAL AND METHODS

Dataset

Records was obtained from data of the stud book of IHDH breed and from the linear type traits information routinely recorded by the National breeder association (anacaitpr.it). The initial datasets consisted in 17,525 evaluations on foals and 7,133 on adults. The records prior to 1992, as well as individuals' data without father and mother, farm, age of birth of foal and mother were eliminated. After editing, 6,691 scores on adults and 11,357 scores on foals were retained for analysis and merged. The final dataset accounted for 18,038 records belonging to 13,938 animals, 4,110 of which scored at both ages. Data were related to an amount of 18,773 animals in the pedigree.

Data consisted in individual type evaluations linearly scored with a 9 point scale system (from 1 unfair, to 5 excellent, including half points) by 33 classifiers in 21 subsequent years of evaluation (i.e. from 1992 to 2013). Evaluations were performed on 11 of the 14 traits routinely recorded in IHDH, because the 3 traits only scored in 30-months animals were not considered for analysis. Evaluation in the adult age also included the final morphological overall score providing a subjective 5-points judgement (from fair to excellent), that was considered in this study.

The traits were classified in the following three class: i) General traits: head size, temperament, frame size, fleshiness, bone incidence; ii) Traits of the trunk: thorax depth, fore diameters, rear diameters, upper line length, upper line direction; iii) Traits of the limbs: rear legs side view, fore feet, rear feet, hind legs back view. The last three traits were not included in this study. A brief description of the traits considered in the present work is reported in Table 1.

Most of traits are used to score the different aspects of horses conformation and appearance individually rather than in combination. Scoring traits individually may more easily reveal differences in conformation between animal compared to situations when different traits are combined (Druml et al., 2008). The two traits combining the scores of single body regions, frame size and fleshiness, evaluate general aspects of individual conformations, i.e. if the proportions of height and trunk are good, and if muscles masses are well developed.

Variance components estimates, heritability, and correlations

Variance components, Heritabilities and genetic correlations between each pair of traits scored at 6 or a 30 months of age were estimated through bi-trait animal models. The non-genetic effects to include within the genetic models were obtained from preliminary ANOVA performed in a previous study (Folla et al., Chapter 4). Data were classified accordingly to the sex and the age of animals at score (in month). The combination of classifier by year was considered in the model of analysis to take into account the effect of each classifier for every year of evaluation, as in Samorè et al. (1997) for Italian Haflinger. A variable called “stud group” was formed to consider the effect of the studs with less than 2 scored foals born from different stallions within a year of evaluation. This was carried out on the basis of geographical position and management (stable, pasture and stable or outdoor), the farm’s production goal (production of foals for heavy draught or fattening), the general prophylaxis on foals (vaccination or not) and the mean value mares’ body condition registered at foals’ evaluation. In this way, groups have been created for neighbouring studs showing similar geographic and management system (Mantovani et al., 2010; Folla and Mantovani, 2013; Folla et al., Chapter 3). Therefore the effect included in the model was a combination between the stud group, the year of birth of foal and the classifier: stud-group-classifier-birth year, or SYC. The genetic model of analysis thus included the fixed effects of SYC, sex, age of individual at scoring, and age of mare at foaling. In bi-trait analyses different effects for SYC and individual age at scoring were considered for 6-months and 30-months animals and all included within the genetic analysis. The final model adopted for bi-trait analyses was written in matrix notation as follows:

$$\begin{vmatrix} \mathbf{y}_1 \\ \mathbf{y}_2 \end{vmatrix} = \begin{vmatrix} \mathbf{X}_1 & 0 \\ 0 & \mathbf{X}_2 \end{vmatrix} \cdot \begin{vmatrix} \boldsymbol{\beta}_1 \\ \boldsymbol{\beta}_2 \end{vmatrix} + \begin{vmatrix} \mathbf{W}_1 & 0 \\ 0 & \mathbf{W}_2 \end{vmatrix} \cdot \begin{vmatrix} \mathbf{p}_1 \\ \mathbf{p}_2 \end{vmatrix} + \begin{vmatrix} \mathbf{Z}_1 & 0 \\ 0 & \mathbf{Z}_2 \end{vmatrix} \cdot \begin{vmatrix} \mathbf{u}_1 \\ \mathbf{u}_2 \end{vmatrix} + \begin{vmatrix} \mathbf{e}_1 \\ \mathbf{e}_2 \end{vmatrix}$$

where $\mathbf{y}_1$ and $\mathbf{y}_2$ are vectors of observations for traits recorded at 6 and at 30 months of age; $\boldsymbol{\beta}_1$ and $\boldsymbol{\beta}_2$ are vectors of systematic fixed effects including the combinations of SYC (2637 levels) at 6 months and at 30 months 1663 levels), the animals sex (male or female), the age of foal at scoring (9 classes, i.e., ≤2, 3, 4, 5, 6, 7, 8, 9, ≥10), the age of adults at scoring (5 classes, i.e., ≤27, 28, 29-32, 33-47, and ≥48 months of age), and the age of mare at foaling (5 classes, i.e., ≤4, 5-6, 7-10, 11-13, ≥1); $\mathbf{p}_1$ and $\mathbf{p}_2$ are vectors of random permanent environmental effects including the individual identities (n=139,38) of both young and adult animals, $\mathbf{u}_1$ and $\mathbf{u}_2$ are vectors of random animal effects accounting for the random additive genetic effect (18,773 levels, as animals in pedigree), and $\mathbf{e}_1$ and $\mathbf{e}_2$ are vectors of the random residual terms. Furthermore, $\mathbf{X}_1$, $\mathbf{X}_2$, $\mathbf{W}_1$, $\mathbf{W}_2$, $\mathbf{Z}_1$ and $\mathbf{Z}_2$ are the corresponding incidence matrices with the appropriate dimensions. The following normal distributions were assumed for random effects: $\mathbf{u}$~$N(0, \mathbf{G}\otimes\mathbf{A})$, $\mathbf{p}$~$N(0, \mathbf{Pe}\otimes\mathbf{I})$, $\mathbf{e}$~$N(0, \mathbf{R}\otimes\mathbf{I})$, where **G**, **Pe** and **R** are the additive genetic, the permanent environmental and the residual (co)variance matrices of order 2x2 including the estimates of the respective variances for trait 1, for trait 2, and their covariance; **A** is the additive genetic relationship matrix; **I** is an identity matrix; and ⊗ is the Kronecker product operator.

Marginal posterior distributions of variances of each trait and (co)variance components between all pairs of the 15 traits were estimated via Bayesian approach and applying the Gibbs sampling algorithm (Sorensen et al., 1994) to run bi-trait analyses with the GIBB3F90 program (Misztal, 2008). Flat priors were used for fixed effects and variance components. Each analysis was run as a single chain of 990,000 cycles with a conservative burn-in of 90,000 iterations. Every 300 cycles a sample was stored for a total of 3000 samples (one every 300 interval point), used for computing posterior means, medians, modes, SD. Post-Gibbs analyses were performed using the Postgibbsf90 program (Misztal, 2008) working on the stored samples. The posterior means and corresponding lower and upper bounds of the 95% highest posterior density (HPD95%) were computed for all heritability estimates and correlations. Heritabilities of traits were calculated as $h^2=\sigma^2_a/\sigma^2_P$, with σ^2_a the additive genetic variance, and σ^2_P the phenotypic variance, as the sum of all the other variance components. The genetic correlations, considered for each pair of traits evaluated both at 6 and at 30 months of age, and for each trait with the overall score, were calculated as $r_a=\sigma_{a1a2}/(\sigma^2_{a1}\cdot\sigma^2_{a2})^{0.5}$, with σ_{a1a2} the additive genetic covariance for the trait pair. Phenotypic correlations were computed as $r_P=\sigma_{P1P2}/(\sigma^2_{P1}\cdot\sigma^2_{P2})^{0.5}$, considering the phenotypic covariance σ_{P1P2} as sum of all estimated covariances (Searle, 1961). Significance of estimates was assumed when the HPD95% interval did not included the zero (e.g., in Battagin et al., 2013).

Response to selection

The evolutionary response to selection $\Delta\bar{z}$ (the predicted change in trait mean after a generation) was calculated in the eleven traits scored both in foals and in adults applying a formula derived from the multivariate breeder's equation (Lande 1979) and considering the economic values of traits (Kause et al., 2015): $\Delta\bar{z}$=i/σI(b'G), where i is the selection intensity, set at 1.755 i.e. selected breeders corresponded to the 10% of population, σI the standard deviation of selection index, G the 12x12 genetic (co)variance matrix (the G matrix), and b the vector of the selection index weights as b=P-1Ga, where P is the 12x12 matrix of phenotypes (the P matrix) and a is a vector of the economic values of traits. The σI was calculated as σI=(bTPb), where bT is the transpose of b. The economic values assigned to the traits were the same that they have in the Total Merit Index (TMI) routinely applied in IHDH genetic improvement (Mantovani et al., 2005; 2010) and expressed as:

TSI=0.25·HS+0.15·TM+0.25·FI+0.15·FD+0.20·RD, where the numbers are the economic values for head size (HS), temperament (TM), fleshiness (FI), fore diameters (FD) rear diameters (RD). The economic values for linear type traits not included in TMI and for the overall score were set at zero in the vector a. The G matrix and P matrix were obtained by the results of the bi-trait genetic analyses performed on each trait pair within linear type traits scored at the same age (within the 6-months or within the 30-months evaluations) and realised for two previous studies (Folla et al., Chapter 3; Folla et al., Chapter 4) on the same datasets of the present one. Finally, values of each zi response to selection for each trait included in the two vectors $\Delta\bar{z}$ obtained for traits scored either in foals or in adults were divided by a generation length L to obtain an annual selection rate $d\bar{z}/dt$. Values of L=2 years and L=4 years were respectively assigned to foals and adults' traits since foals are valued earlier in individuals life permitting a faster genetic improvement.

5.3 RESULTS

Dataset

Some descriptive statistics about the traits considered in the study have been already reported in two previous works, respectively focused on the linear type evaluations in foals or in adults (Folla et al., Chapter 3; Folla et al., Chapter 4). Briefly, the average values of linear type traits phenotypes (data not shown) ranged from 2.5 points (rear legs side view valued at 30 months) to 3.5 (thorax depth, same value at each age), and standard deviations, ranged from 0.4 to 0.6. Most traits showed a medium value closed to 3 points, whereas the overall score repored a lower mean value of 2.0±0.8 points.

About the skewness, most traits showed lower values of asymmetry (data not shown), resulting close to a normal distribution. Some exceptions were found for upper line direction and rear legs side view in foals, and upper line direction in 30-months animals, all reporting a negative skew due to a greater frequency of high scores. High values of kurtosis (close to 2 or greater; data not shown) were found for upper line direction at both ages and for bone incidence at 30 months (despite the distribution at 6 months resulted a bit platycurtic).

Variance components estimates and heritability

Posterior mean and 95% highest posterior probability density (95%HPD) intervals for additive genetic, permanent environmental and residual variances, and for heritabilities of all traits under study are reported in Table 2, also including the threshold to obtain heritability estimates with P>0.95 (h2>95%). Statistics were based on the marginal posterior density estimations obtained using the pooled samples of 3000 Gibbs chains obtained in bi-trait analyses. The 95%HPD interval of confidence for the genetic variance is lower than for the permanent environmental and residual variances, both reporting high values. The 95%HPD interval for all the σ2Pe estimates overlapped zero or was very close to it, meaning a lack of statistical relevance for the σ2Pe component. This may be because the repeated data of individuals, when present, were from different datasets (6-months and 30-months data) and did not share any environmental variance components apart the sex. All the variance of individual data was probably included within the additive genetic component, as if it came from a dataset with single individual records.

Heritability values ranged in the foals from h^2=0.07 (rear legs side view), to h^2=0.36 (muscularity), and intervals of confidence for posterior density estimates measured about 10 percentage points for all traits. In the adults not greater differences than in foals were found in the heritabilities of traits, that ranged from h^2=0.04 (upper line direction) to h^2=0.40 (frame size), and the 95%HPD intervals showed a similar size. The lowest heritabilities in both foals and adults' traits were found for rear legs side view, and upper line length and direction, all traits related to the conformation correctness and with intermediate optima. A stabilizing selection is probably occurring on these traits (Gibson and Bradley, 1974). The heritability of traits included in the TMI and scored at 6 months ranged from h^2=0.20 (Temperament) to h^2=0.36 (Fleshiness, but closer values were reported also for Head size and Fore diameters), and similar estimates were found at 30 months with the exception of the h^2 for fleshiness, 11 percentage points lower than in the 6-months estimate. The other different h^2 estimates in foals and adults' traits were reported for frame size, 14 percentage points greater in 30-months traits. Then, the overall score showed a medium heritability of 0.30 with a similar 95%HPD than in the other traits.

Phenotypic and genetic correlations

Genetic and phenotypic correlations among same traits scored at 6 months and at 30 moths, and related 95% highest posterior density intervals, are reported in Table 3. Most of the permanent environmental covariances presented values not different from zero, except for head size, bone incidence and upper line length, whereas all the residual covariances, reporting high values of 95%HPD, did not differed from zero. That is probably because traits were measured at different times (diverse ages), and environmental covariances, when present, were then accounted within the σPe12 when significantly different from zero. The genetic covariances were all different from zero and resulted about twice than in the other traits for head size, frame size and fore diameters. Genetic correlations among the 11 eleven traits recorded at 6 and at 30 months were high and all different from zero, ranging from r_g=0.55 to r_g=0.92. The lowest values were found for fleshiness r_g=0.66 (95%HPD=0.51; 0.80) and thorax depth r_g=0.55 (95%HPD=0.36; 0.74), maybe due to the high genetic correlation between thorax depth and fleshiness (Folla et al., Chapter 4), and the selection for fleshiness occurring on foals and probably shrinking the genetic variability of trait at adult age. The highest values of r_g were found for frame size, r_g=0.92 (95%HDP=0.84; 1.01), upper line direction, r_g=0.88 (95%HDP=0.62; 1.13) and head size, r_g=0.87 (95%HDP=0.77; 0.96). The genetic correlations of traits involved in TSI ranged from r_g=0.66 (fleshiness) to r_g=0.87 (head size).

The phenotypic correlations ranged from -0.16 (upper line direction) to 0.47 (head size), but they were all not different from zero.

Table 4 reported the genetic and phenotypic correlations among the overall score and all the other 11 traits scored at 6 and 30 months. The correlations between overall score and 30-months traits were already performed in a previous study (Folla et al., Chapter 4) via AIREML estimation (Misztal et al., 2008) and here reported with their standard errors (Lynch and Walsh, 1998).

The highest value of r_g estimates among overall score and traits scored on 6-months animals was found for frame size, r_g=0.80 (HDP95%=0.66; 0.94), while the lowest value was for bone incidence r_g=0.02 (HDP95%=-0.19; 0.23). Folla et al (Chapter 4) reported some different values of genetic correlations: most of traits reported higher values than in r_g of overall score and 6-months traits, except for bone incidence, r_g=0.13±0.108, head size, r_g =0.45±0.069 and temperament, r_g=0.47±0.076. While upper line length, direction and rear legs side view the values showed little differences. The highest increment of r_g values was found for frame size, r_g=0.85±0.034. Bone incidence and correctness traits upper line length and direction, and rear legs side view showed with overall score genetic correlations not different from zero when recorded both at 6 and 30 months.

Most phenotypic correlations with overall scored performed on traits recorded at 30 months were higher than r_P of overall score and 6-months traits, except for bone incidence and rear legs side view, reporting unchanged correlations. All the phenotypic correlations on foals did not differed from zero whereas the ones calculated on 30-months traits were significant apart upper line length. The difference may be due to the different algorithm used for estimations, and to the fact that correlations of overall score and 30-months traits are performed on the same individuals.

Response to selection

The values of multitrait evolutionary response to selection are reported in Table 5. The response has been estimated for all 11 traits scored both in foals and in adults and on the overall score by alternatively considering the current selection of traits at 6 months and a possible selection at 30 months. The current economic values of traits within the TMI have been considered together to measure the strength of selection alternatively acting on foals and on adults traits. The indirect selection occurring on the traits not included in the TMI has been also represented by assigning an economic values of zero to these traits. The evolutionary response to selection per generation $\Delta\bar{z}$ has shown almost little differences within pairs of the same trait scored at different ages (e.g., temperament reported $\Delta\bar{z}_6$ =0.126 points and $\Delta\bar{z}_{30}$ =0.136 Some more differences appear when the annual genetic rate of traits ($d\bar{z}/dt$) is considered: a greater annual variation is shown for the traits included in the TMI and recorded on foals, reporting an average value of $d\bar{z}/dt$ =0.101±0.029 vs. an average $d\bar{z}/dt$ =0.067±0.017 on adults. Not included in selection index but positively correlated at genetic level with traits in TMI, also frame size and thorax depth showed positive annual genetic rates both in traits recorded in foals ($d\bar{z}/dt$ =0.149 and $d\bar{z}/dt$ =0.076, respectively) and adults ($d\bar{z}/dt$ =0.058 and $d\bar{z}/dt$ =0.033, respectively). Annual genetic rates close to zero both at 6 and at 30 months were shown for bone incidence, negatively correlated with most of the other traits (Folla et al., Chapter 3; Folla et al., Chapter 4), and for upper line length and direction and rear legs side view, all traits with intermediate optima, and in which a trend of zero and centred in the mean value of breeding values is preferred. Finally, positive genetic rate have been reported for the indirect selection in overall score, when considered both with foals ($d\bar{z}/dt$=0.179) and adult ($d\bar{z}/dt$=0.084) traits.

5.4 DISCUSSION

The improvement of genetic value of traits has been a constant objective for breeders over years for providing valuable quality animals to the meat industry. Genetic parameters estimates are an important requisite for sound improvement programmes since they determine selection criteria and the potential for genetic improvement. The quantitative analysis of the evolution of a suite of traits requires two sets of parameters, the heritability of the traits (h^2) and the genetic correlation (r_g) between each pair, occurring since some gene may effect more than one trait, therefore the traits can be genetically correlated (Falconer and McKay, 1989).

In selection programs, the emphasis to be placed on the various traits can depend, in part, on the genetic correlations among them (Roff, 1996). In addition, genetic correlations can be used to predict what is expected to happen to traits not used in selection as a result of an indirect selection on them (Falconer and McKay, 1996). This effect on traits not directly used in selection programs is referred to as correlated response to selection (MacNeil et al 1985). The knowledge of genetic correlations is needed for multiple trait evaluation of individuals, and for the prediction of the correlated responses to selection (Falconer and MacKay, 1996). The measurement of genetic correlations between traits is commonly performed in applied animal and plant breeding, and in the evolutionary genetics of natural populations. In all fields it is desirable to obtain estimates of genetic correlations in order to understand the hereditary constraints that influence the response of the vector of phenotypes to natural or artificial selection (Lande and Price, 1989).

Genetic response to selection (Falconer and McKay, 1996) is usually measured in units per year rather than units per generation, because the intention of breeders is usually to increase the mean value of a trait as quickly as possible in the favoured direction. Genetic response firstly depend on the intensity of selection and on the additive genetic variation of selected traits. In a multivariate framework, the knowledge of all the genetic and phenotypic (co)variances (G matrix and P matrix) among traits directly or indirectly selected is mandatory for a proper study of the occurring selection processes (Lande, 1979).

Furthermore, the common assumption that G matrix of focal traits is stable over space and time has been widely argued (e.g., Steppan et al., 2002), and important changes in heritability of traits may be observed at different ages of individuals (Charmantier et al., 2006). Some of these changes may be due to maternal effects, occurring when the phenotype of the mother affects the one of the offspring (Falconer and McKay, 1996). Some other age-specific changes may be a consequence of selection, if selection differently acts on the phenotypic variance of a trait (Charmantier et al., 2006). This may occur when selection directly act on another trait and reduces the environmental variance for that trait, therefore increasing its heritability and producing a correlated response on the other correlated traits (Merilä et al., 2001).

Some variations in the heritabilities of traits observed in foals and adults of the present study may be due to the action of selection directly operating on traits included in the selection index, or TMI, and leading to a correlated response to selection on the other traits, genetically correlated with them. The heritability of traits and their selection optima (if the positive extreme value or intermediate) also concur in determining the evolutionary response to selection of traits, and their genetic values expressed at different ages.

The comparison of the genetic parameters obtained in the present study with estimates in other breeds it’s not simply, especially because studies about correlations on traits recorded in different times are scarce (see the Introduction). Furthermore, some of the traits of the study, i.e., traits related to meat production, as fleshiness, not been very well studied in comparison with other species, and some comparison can be made with beef cattle breeds, having the same main selection goal of IHDH that is meat. In general, it is difficult to compare evaluations of conformation traits derived from several breeds and scoring system, because of serious differences in breeding goals and the way of scoring (Druml et al., 2008).

In the present study the comparison of the heritability of traits evaluated in different moments of the life has reported that only two traits showed differences: the heritability of frame size increased from h^2=0.26 in foals to h^2=0.40 to adults, while fleshiness decrease from h^2=0.36 estimated in foals to h^2=0.25 on adults. In the other traits similar h^2 values found. The differences in fleshiness h^2 could be due to the selection performed on young foals that could lead to have individuals with similar (and high) genetic value at adult age. Furthermore, the greater heritable variance in frame size could be due to a genetic variability for growth among animals that may express later than in the first months of life.

Comparing the results with other studies evaluating the heritability of traits at young and adult age, some considerations can be performed. Teegen et al. (2006) reported a h^2 value of type in Trakehner horse population, comparable with frame size in the IHDH, of h^2=0.46 in foals and higher values (h^2=0.58) in the mares. In the same study, the body conformation reported a lower value of h^2=0.29 in the foals and higher value of h^2=0.47 in the mares. For overall impression Teegen et al. (2006) reported a h^2=0.40 in the foals and h^2=0.70 in the mares, in both case they are higher values than the heritabilities found in the IHDH for the overall score at adult age (h^2=0.30). Schöpke et al., (2013) studied in German sport horse different traits in foals, broodmares and mares. Foals and broodmares were evaluated through inspection, and mares using performance test. Comparing head size of IHDH with similar traits in German horse sport, a similar value of h^2=0.50 was found in foals e broodmares. Again, the conformation traits showed a value of h^2=0.24 in the foals and h^2=0.43 in the broodmares. Jönsson et al. (2014), in their study on Danish warmblood, found value for head (h^2=0.35) similar to estimate in IHDH. Viklund et al., (2008) considered in his study genetic evaluations on different ages and in three different periods in Swedish Warmblood Horses. The study reported a value of h^2=0.58 for head-neck-body in foals, while in adults the heritability value ranged from h^2=0.21 to h^2=0.25 considering three different periods (up to 4 years, up to 9 years and lifetime). Suontama et al. (2011), found for body conformation heritability values of h^2=0.31 and h^2=0.46 for foals of Finnhorse and Standarbred trotters, and h^2=0.10 and h^2=0.13 for adults. The heritability of temperament in the present study was similar for both ages of IHDH animals, and Suontama et al., (2011) also found similar values in his study for the two breeds they considered (Finnhorse and Standarbred trotters, h^2=0.18 and h^2=0.11).

Comparing traits related to muscularity with estimates in cattle breeds, Mazza et al. (2015) in Valdostana Red Pied and Black Pied found for muscularity, front muscularity and rump width heritability values ranging from h^2=0.08 to h^2=0.26.

Higher values, ranging from h^2=0.25 to h^2=0.36 considering both ages, were found in this study.

The overall score, trait evaluated only at adult age, showed a heritability of 0.30. Molina et al (1999) found a value of h^2=0.25 for total score in Andalusian horse, whereas Bhatnagar et al. (2011) found an heritability of 0.55 for the same trait in foals of American Sporthorse. Schroderus et al. (2010) reported heritabilities for the overall score of h^2=0.32 for Finnhorse and h^2=0.34 for Standardbred foals. Jönsson et al. (2014) found a value of h^2=0.48 for overall conformation in Danish Warmblood Horse. Suontama et al., (2011) reported for overall grade a value of h^2=0.31 in the foals Finnhorse trotter and h^2=0.36 in Standardbred foals.

Different levels of correlations were found in conformation traits scored at different ages in horse breeds. Schöpke et al. (2013) found a genetic correlation of r_g=0.82 between conformation scores evaluated in foals and adults of German Sport Horse, higher than found in this study (r_g=0.66). Viklund et al. (2008) found a correlation between head-neck-body scores evaluated in Swedish Warmblood horse foals and adults of r_g =0.96, higher than found in the present study for head size (r_g =0.87). The Authors also reported a value of r_g =0.97 for total conformation. Suontama et al. (2011) reported in their study about Finnhorse trotter and Standardbred trotters some estimations of genetic correlations between foals and studbook horses. About Finnhorse trotter, the genetic correlation between body conformation scores evaluated at the two ages was of r_g =1.00, and similar levels were found between leg stances scores (r_g =0.94).

About the genetic correlations of traits recorded at different ages with the overall score summarizing the morphological assessment of a breed, Teegen et al., (2006) found high values of r_g =0.90 and r_g =0.89 between body conformation and overall impression respectively in foals and mare of Trakehner horse, a bit higher than estimates in foals (r_g =0.80) and adults (r_g =0.85) IHDH for frame size found in this study. The high genetic correlations obtained in both studies indicate that the traits scoring the conformation (the frame) are of main importance for the final judgement of an individual. Another trait important for the final judgment in a breed is type. The correlation found in Trakehner population between type and overall impression was r_g=0.72 in foals and r_g =0.78 in adults (Teegen et al., 2006). Schöpke et al. (2013) found in foals of German sport horse a genetic correlation of r_g =0.89 between overall impression, only scored in mares, and conformation, consistent with Teegen et al. (2006) and this study. Furthermore, the overall impression showed a correlation with head of r_g=0.77, a bit higher than in IHDH. The overall grade showed in Finnhorse and Standardbred trotters (Suontama et al., 2011) a different genetic correlation with movement in the two breeds, of r_g=0.36 (Finnhorse trotter) and r_g=0.72 (Standardbred trotter) and a r_g=0.64 to body conformation in both breeds.

The different genetic correlations among traits occurring in different ages of animals may suggest that different responses to selection may be realized under the same selection pressures. In addition to the variations in the genetic structure of traits (the G matrix) occurring at different ages, the evolutionary response may also depend by the age itself in which the selective process is realized (Falconer and McKay, 1996). A selection occurring early in individual life may provide a faster response than selecting at older age (Hill, 1974). This consideration is a basis of breeding programs in a number of livestock species, such as in cattle, in which a faster genetic improvement is realized accounting for difference ages at reproduction for males and females (Falconer and McKay, 1996).

Genetic correlations among traits and predicted selection rate of the present study have shown that since a similar genetic structure is present in foals and adult of IHDH, and higher genetic correlations occur among overall score and the other traits, a routinely selection accounting for traits scored in adults and not in foals is feasible. Anyhow, the younger age at evaluation allows a faster genetic improvement, and further considerations about cost savings or speeding up genetic trend will allow to decide which individuals, foals or adults, should be the best target for selection.

5.5 CONCLUSIONS

This study has considered the genetic relationships of linear type traits routinely scored in Italian Heavy Horse breed at 6 months or at 30 months of age. The magnitude of heritability in both cases was medium low: the higher values were found for traits correlated to muscularity (fleshiness, fore and rear diameters), and the lower in traits related to conformation correctness (upper line length and direction, rear legs side view. All traits involved in Total Merit Index (head size, temperament, muscularity, fore and rear diameters) have reported similar heritability in foals and adults' traits, ranging in foals from h^2=0.20 to h^2=0.36, and in adults from h^2=0.21 to

h^2=0.32. The only relevant differences in h^2 were found for frame size and fleshiness, respectively 14 percentage points higher and 11 percentage points lower in adults. That's may be due to the genetic variability in growth, and to the selection for muscularity occurred in foals. The genetic correlations among the same traits scored at different ages are resulted high, and ranged from r_g=0.55 (thorax depth) to r_g=0.92 (frame size). Genetic correlations of traits scored at 30 months with overall score were generally higher than the correspondent r_g of overall score with traits scored at 6 months. All these considerations indicate that it is possible to select the animals at adult age, and therefore reducing the costs for selection (less animals are valued at adult age). Notwithstanding, the faster selection rate observed in young foals due to the younger age at selection suggests that, in order to speed up the genetic trend of traits, the selection on young foals still remain the best solution.

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***Table 1**. Description of the 11 linear type traits in IHDH horses evaluated at 6 and 30 months of age, and of the overall score, only evaluated at 30 months.*

Trait	Abb.	Description
Head size	HS	Volume of head, from heavy to light, eyes expression, neck muscle, animal posture
Temperament	Te	Response to stimuli, impulsion to the movement, amplitude of steps, regularity of pace
Frame size	FS	Trunk-stature proportion, as weighted sum of diameters, thorax depth and height
Fleshiness	Fl	Development from poor to excellent of muscularity of croup, thigh, buttock, loins, wither
Bone incidence	BI	Bone frame, from fine to heavy, in relation to muscularity, and head volume
Thorax depth	TD	Height of the thorax, valued from little to large
Fore diameters	FD	Size of the chest, from narrow to wide; scored to evaluate the anterior skeletal size
Rear diameters	RD	Size of the croup, from narrow to wide; scored to evaluate the space for muscle masses
Upper line length	UL	Overall length of the back-loins; from short to long; intermediate optimum
Upper line direction	UD	Presence of curvature or kyphosis; from kyphotic to curved; intermediate optimum
Rear legs side view	LS	Angle between the base of the hock and the shin; from sickle to straight; interm.optimum
Overall score	OS	Final morphological judgement, from fair to excellent

***Table 2**. Variance components and heritability with the respective 95% highest posterior density interval (95%HPD, in brackets), for linear type traits scored in foals and adult IHDH horses and for the overall score. The heritability threshold with P>0.95 is also reported. Significant estimates were bolded.*

	Foals					30 months				
	σ_a^2	σ_{pe}^2	σ_r^2	h^2	h^2>95%	σ_a^2	σ_{pe}^2	σ_r^2	h^2	h^2>95%
Head size	**11.49** **(9.56; 13.41)**	4.28 (-6.08; 14.64)	**17.81** **(7.43; 28.18)**	**0.35** **(0.29; 0.40)**	0.30	**10.5** **(8.56; 12.44)**	**10.29** **(0.49; 20.09)**	**11.85** **(2.04; 21.66)**	**0.32** **(0.27; 0.37)**	0.14
Temperament	**4.19** **(3.17; 5.21)**	2.92 (-5.12; 10.95)	**14.44** **(6.41; 22.47)**	**0.20** **(0.15; 0.24)**	0.16	**4.69** **(3.49; 5.88)**	7.13 (-0.19; 14.45)	**11.01** **(3.68; 18.34)**	**0.21** **(0.16; 0.26)**	0.13
Frame Size	**6.38** **(4.99; 7.78)**	4.69 (-4.31; 13.69)	**14.18** **(5.23; 23.13)**	**0.26** **(0.20; 0.31)**	0.21	**14.63** **(12.27; 16.99)**	8.29 (-0.15; 16.73)	**12.48** **(3.97; 20.98)**	**0.40** **(0.34; 0.46)**	0.13
Fleshiness	**8.01** **(6.48; 9.54)**	3.71 (-4.47; 11.88)	**12.03** **(3.79; 20.28)**	**0.36** **(0.30; 0.41)**	0.31	**5.45** **(4.16; 6.73)**	7.15 (-0.58; 14.89)	**9.21** **(1.49; 16.92)**	**0.25** **(0.20; 0.31)**	0.11
Bone Incidence	**2.07** **(1.37; 2.77)**	6.23 (-2.14; 14.61)	7.66 (-0.71; 16.03)	**0.12** **(0.08; 0.17)**	0.08	**1.71** **(1; 2.43)**	**4.58** **(0.03; 9.13)**	**6.75** **(2.18; 11.32)**	**0.14** **(0.08; 0.19)**	0.10
Thorax depth	**3.3** **(2.38; 4.21)**	3.16 (-4.51; 10.83)	**12.15** **(4.45; 19.85)**	**0.18** **(0.13; 0.22)**	0.13	**4.56** **(3.32; 5.8)**	**6.90** **(0.42; 13.37)**	**8.99** **(2.57; 15.4)**	**0.22** **(0.16; 0.27)**	0.10
Fore Diameters	**9.27** **(7.64; 10.89)**	4.45 (-4.57; 13.48)	**13.52** **(4.45; 22.6)**	**0.35** **(0.29; 0.40)**	0.30	**9.11** **(7.3; 10.92)**	**9.46** **(0.76; 18.16)**	**10.17** **(1.3; 19.04)**	**0.32** **(0.26; 0.37)**	0.09
Rear Diameters	**7.21** **(5.72; 8.69)**	3.75 (-5.00; 12.50)	**13.36** **(4.53; 22.19)**	**0.30** **(0.25; 0.36)**	0.26	**6.73** **(5.34; 8.12)**	**7.96** **(0.02; 15.91)**	**8.98** **(1.05; 16.91)**	**0.27** **(0.22; 0.33)**	0.08
Upper Line Length	**1.53** **(0.9; 2.17)**	5.98 (-1.62; 13.58)	**7.74** **(0.16; 15.32)**	**0.10** **(0.05; 0.14)**	0.06	**1.88** **(1.16; 2.6)**	5.48 (-0.87; 11.82)	**9.15** **(2.79; 15.5)**	**0.11** **(0.06; 0.15)**	0.05
Upper line Direction	**0.43** **(0.16; 0.7)**	1.46 (-1.77; 4.68)	**5.5** **(2.28; 8.73)**	**0.06** **(0.02; 0.10)**	0.03	**0.47** **(0.19; 0.76)**	2.89 (-0.91; 6.68)	**5.39** **(1.56; 9.23)**	**0.04** **(0.01; 0.08)**	0.04
Fore Legs Side View	**0.86** **(0.44; 1.29)**	2.92 (-2.56; 8.4)	**8.52** **(3.04; 14.00)**	**0.07** **(0.03; 010)**	0.04	**2** **(1.23; 2.76)**	5.03 (-0.93; 10.99)	**8.82** **(2.85; 14.79)**	**0.13** **(0.08; 0.18)**	0.05
Overall Score	-	-	-	-	-	**12.86** **(10.37; 15.35)**	**12.13** **(0.09; 24.17)**	**16.14** **(4.17; 28.12)**	**0.30** **(0.25; 0.36)**	0.26

σ_a^2=additive genetic variance; σ_{pe}^2=permanent environmental variance; σ_r^2=residual variance; h^2=heritability.

Table 3. *Covariances and phenotypic and genetic correlations with the respective 95% highest posterior density interval (95%HPD, in brackets), for each pair of the same linear type trait either scored in foals (6 months of age) or in adult (30 months of age) IHDH horses. Significant estimates were bolded.*

Trait	σ_{pe12}	σ_{a12}	σ_{r12}	r_g	r_p
Head size	**2.27 (0.87; 3.67)**	**9.48 (7.97; 11)**	2.70 (-16.1; 21.49)	**0.87 (0.77; 0.96)**	0.47 (-0.12; 1.07)
Temperament	0.04 (-0.91; 1)	**3.40 (2.6; 4.2)**	-4.17 (-21.53; 13.2)	**0.77 (0.6; 0.95)**	-0.03 (-0.89; 0.82)
Frame Size	-0.23 (-1.56; 1.09)	**8.91 (7.52; 10.3)**	3.22 (-14.54; 20.99)	**0.92 (0.84; 1.01)**	0.42 (-0.2; 1.04)
Fleshiness	0.35 (-0.79; 1.49)	**4.33 (3.27; 5.39)**	-0.6 (-14.91; 13.71)	**0.66 (0.51; 0.8)**	0.20 (-0.46; 0.86)
Bone Incidence	**1.26 (0.58; 1.93)**	**1.6 (1.09; 2.12)**	2.28 (-6.64; 11.19)	**0.86 (0.67; 1.05)**	0.38 (-0.27; 1.03)
Thorax depth	0.54 (-0.38; 1.46)	**2.11 (1.34; 2.89)**	1.03 (-13.56; 15.63)	**0.55 (0.36; 0.74)**	0.20 (-0.62; 1.02)
Fore Diameters	0.41 (-0.78; 1.6)	**7.17 (5.92; 8.42)**	3.25 (-11.77; 18.26)	**0.78 (0.68; 0.89)**	0.41 (-0.14; 0.97)
Rear Diameters	-0.12 (-1.21; 0.98)	**5.63 (4.54; 6.72)**	-2.89 (-17.24; 11.46)	**0.81 (0.7; 0.93)**	0.12 (-0.53; 0.77)
Upper Line Length	**1.75 (1.01; 2.49)**	**1.42 (0.91; 1.93)**	3.27 (-6.2; 12.74)	**0.84 (0.65; 1.03)**	0.44 (-0.2; 1.09)
Upper line Direction	0.25 (-0.2; 0.7)	**0.38 (0.18; 0.59)**	-1.95 (-9.37; 5.47)	**0.88 (0.62; 1.13)**	-0.16 (-1.15; 0.83)
Fore Legs Side View	0.47 (-0.15; 1.08)	**1.06 (0.64; 1.49)**	3.91 (-7.35; 15.18)	**0.82 (0.59; 1.05)**	0.41 (-0.44; 1.27)

σ_{pe12}=permanent environmental covariance between traits 1 and 2; σ_{a12}=additive genetic covariance; σ_{pe12}=permanent environmental covariance between traits 1 and 2; σ_{r12}=residual covariance; r_g=genetic correlation; r_p=phenotypic correlation;.

Table 4. *Phenotypic and genetic correlations with the respective 95% highest posterior density interval (95%HPD), or standard error (both in brackets) for each pair of overall score with linear type traits scored in foals and in adult IHDH horses. Significant estimates were bolded.*

Trait	Overall Score vs foals single traits		Overall Score vs adults single traits[1]	
	r_g	r_p	r_g	r_p
Head size	**0.66 (0.51; 0.80)**	0.20 (-0.46; 0.86)	**0.45 (0.069)**	**0.31 (-0.014)**
Temperament	**0.58 (0.43; 0.74)**	-0.06 (-074; 0.62)	**0.47 (0.076)**	**0.30 (-0.014)**
Frame Size	**0.80 (0.66; 0.94)**	0.18 (-0.40; 0.77)	**0.85 (0.034)**	**0.58 (-0.01)**
Fleshiness	**0.29 (0.16; 0.43)**	0.12 (-0.52; 0.75)	**0.61 (0.061)**	**0.46 (-0.012)**
Bone Incidence	0.02 (-0.19; 0.23)	-0.08 (-0.92; 0.76)	-0.13 (0.108)	**-0.09 (-0.015)**
Thorax depth	**0.46 (0.29; 0.63)**	0.06 (-0.71; 0.83)	**0.72 (0.060)**	**0.41 (-0.013)**
Fore Diameters	**0.52 (0.39; 0.65)**	0.25 (-0.38; 0.88)	**0.70 (0.049)**	**0.52 (-0.011)**
Rear Diameters	**0.44 (0.31; 0.58)**	0.11 (-0.59; 0.82)	**0.77 (0.047)**	**0.54 (-0.011)**
Upper Line Length	0.20 (-0.02; 042)	-0.12 (-0.94; 0.70)	0.19 (0.122)	0.01 (-0.015)
Upper line Direction	0.27 (-0.04; 0.59)	-0.22 (-1.15; 0.72)	0.23 (0.201)	**0.04 (-0.014)**
Rear Legs Side View	0.08 (-0.17; 0.32)	0.30 (-0.57; 1.18)	0.05 (0.110)	**0.31 (-0.014)**

1 from Folla et al., Chapter 4 of this thesis

Table 5. *Economic weight of the 11 linear type traits scored both in foals and in adult IHDH and predicted response to selection and annual selection rate calculated by assigning to each trait the respective economic value, and by applying a multivariate breeders' equation (Lande 1979)*

Trait	Economic weight	Response to selection		Annual selection rate	
		Foals	Adults	Foals	Adults
Head size	0.25	0.209	0.274	0.105	0.069
Temperament	0.15	0.126	0.136	0.063	0.034
Frame Size	0	0.297	0.231	0.149	0.058
Fleshiness	0.25	0.197	0.298	0.099	0.075
Bone Incidence	0	-0.034	0.005	-0.017	0.001
Thorax depth	0	0.151	0.131	0.076	0.033
Fore Diameters	0.15	0.256	0.329	0.128	0.082
Rear Diameters	0.20	0.222	0.296	0.111	0.074
Upper Line Length	0	0.041	0.001	0.021	0.000
Upper line Direction	0	0.002	0.023	0.001	0.006
Fore Legs Side View	0	-0.024	0.033	-0.012	0.008
Overall Score	0	0.358	0.336	0.179	0.084

CHAPTER 6

GENETICS OF LIFETIME REPRODUCTIVE PERFORMANCE IN ITALIAN HEAVY DRAUGHT HORSE MARES

6.1 INTRODUCTION

Fertility has a well-recognized role in animal production for its implication on the economic efficiency of any productive system, independently from the reared specie or the sector considered (Stott et al., 1999; Langlois and Blouin, 2004; Bormann and Wilson, 2010). During the last decades, many studies have been focused on cattle fertility as a breeding goal both in the beef (Meyer et al., 1990; Phocas et al., 1998; Robinson et al., 2008) or, more recently, in the dairy sector (Liu et al., 2008; VanRaden at al., 2014; Dezetter et al., 2015). However, fewer studies on fertility have been carried out in horse as compared to cattle, although horse fertility has been indicated as one of the strategic research aims within the EU by the Fabre Technology Platform (2011). Most researches in the horse industry has dealt mainly with the optimization of subfertility problems that occurs both in stallions (Vidament, 2005) and mares (Gillaume et al. 2006), and extensive reviews have been produced aiming at investigating relationship between reproduction success and management (Davis Morel, 2008), nutrition (Ellis et al., 2006) or genetics (Giesecke et al., 2010). In addition, retrospective studies carried out at population level analyzing reproduction layouts (Hemberg et al., 2004; Mantovani et al., 2013) or on factors affecting horse births (Langlois & Blouin, 2004) are available in literature for this specie. However, little literature is available on the use of fertility traits in horse for breeding purposes as compare to cattle, particularly with beef cattle, that shares with horses the common characteristics of a strong seasonality, in spite of a different reproductive efficiency, timing of ovulation, insemination protocols and gestation length (Davis Morel, 2008). Following the review of Cammack et al. (2009) focused on breeding for in beef cattle, there is no easy definition for this trait, depending on a large amount of factors affecting the reproduction success in both sexes. In addition, among different fertility traits considered, many of them have shown a generally low heritability (Cammack et al, 2009). Mayer et al. (1990), distinguishing between traits pertaining to the lifetime of an individual or repeated over subsequent breeding seasons, showed that the latter traits were generally characterized by a slightly lower heritability. From a genetic point of view, Ponzoni et al. (1992) confirmed the better results obtainable through the use of a lifetime fertility trait as the calving rate (i.e., the efficiency of a female), in comparison to the calving date (i.e., the day of the year in which a cow calves). However, some deficiencies can be attributable to calving rate because of animals have records after a number of calving (Meyer at al., 1990; Ponzoni et al., 1992), delaying selection choices and reducing genetic progress. For this reason but also for the greater economic value of the calving date, this latter trait has been suggested as more suitable for analyzing fertility in beef cattle. However, the greater economic value produced by an early parturition due to the greater weaning weight is not always perceptible in horse breeding, where foals sold at the end of the breeding season are not necessarily evaluated on the basis of weight, but on forelimb conformation and health status (Santschi et al., 2006). However, coldblood horses sold for meat production have more in common with the beef cattle production system, and the weight of weanlings could be considered attractive for animal breeding decisions because of its clearly identifiable economic value. On the other hand, the use of foaling date (i.e., the day of the year on which the mare foals) as possible fertility trait in mares has some possible drawbacks. For instance, some limitation for a correct comparison of mares' reproductive performances could be due to the fact that the breeding seasons do not start at population level on the same date. Moreover, the joining period (i.e., start of the breeding season), is not well documented in many situations, lacking pasture rearing systems. In addition, the gestation length in mares can easily lead to unwanted absence of conception (i.e., open mares; Mantovani et al., 2013), increasing the possible number of missing information on subsequent reproductive seasons. Therefore, a lifetime fertility traits based on the reproductive success of mares could be probably more desirable and of easier use and comprehension for breeders. Mimicking Meyer et al. (1990), a lifetime foaling rate (LFR) could be defined as the number of foals produced by a mare divided by the number of opportunities to do so. Such trait could well represent a measure of the efficiency of reproduction of a mare, although with known limits, i.e., possible asymmetrical distribution due to the proportion variable, or need of a sufficient number of available records per mare. Additionally, longer lifetime can increases opportunities of foaling but also the chance of failure, and older mare could express lower ratios values than younger animals. Moving from these points, this study has aimed at analyzing lifetime reproductive performance in Italian Heavy Draught Horse (IHDH) mares, and particularly at i) identifying and validate a phenotypic variable useful to define a measure of IHDH mares' lifetime fertility, and ii) to analyze the genetic component, genetic trends and rank correlations between

EBVs obtained with 2 different method to estimate a LFR phenotypic value at a given endpoint in IHDH mares by 2 arcsin transformation of the same LFR variables, because of the known possible problems due to the use of a ratio variable.

6.2 MATERIAL AND METHODS

All data used in the study were obtained from the reproductive events officially registered at the stud-book of the Italian Heavy Draught Horse (IHDH) breed, whose characteristics have been described elsewhere (Mantovani et al., 2005; 2013). The study was carried out in two steps. In the first step a training dataset was used in order to develop and compare alternative predictive methods to estimate the foal production at the 6th reproductive season in order to obtain a lifetime foaling rate (LFR) expressed ad number of produced foals produced after 6 reproductive season divided by the opportunities, i.e., 6. The choice of the 6th reproductive season was established as the endpoint representing a successful reproductive career for a IHDH mare, i.e. at 9-10 years of age. In a second step, a full data set was obtained in order to estimate the variance components for the lifetime foaling rate obtained by applying the predictive methods developed at step 1 for incomplete career as respect to the 6th reproductive season. From this latter dataset EBV were also obtained and both genetic trends and rank correlations were compared considering the 4 different expression of the LFR depending on the use of predictive coefficients or equations for incomplete career, and from the 2 arcsin transformed LFR (i.e., arcsin LFR from coefficients or from equations predictive methods). The characteristic of both datasets used in the study are reported in table 1.

Training dataset and analysis

The training dataset contained all reproductive events available from the studbook database for mares born after 1990. To enter the dataset, mares were required to hold a minimum of 6 subsequent registered reproductive seasons, to belong to environmental units with at least 2 observations (group of farm-studs in the same geographical area and common rearing system by year of birth), and to have both parents known. The registered reproductive season accounted for three different events: foaling, abortion or involuntary absence of conception (i.e., open mares) in subsequent calendar years. The choice of the 2 mares in the same environmental unit rather than within stud-farm was necessary because of the small size of stud-farms in the population (i.e., the studbook average number of mares/stud farm is 3.3). The data editing encompasses also the discard of mares that had a first registered events when aged < 3 years or > 4 years or mares with an interval between foaling < 11 or > 17 months as previously used in Mantovani et al. (2013). At the end of the editing process, 1,487 mares were retained for the subsequent analysis that consisted in obtaining a set of predictive coefficients (through the GLM procedure of SAS), or equations (by means of the REG procedure of SAS, SAS Inst. Inc., Cary, NC) allowing the estimates of the no. of foals produced at the 6th reproductive season depending on the basis of i) the previous no. of foals after 3, 4 or 5 reproductive seasons, and ii) the age at first foaling (3 or 4 years; Table 1). A lifetime foaling rate (LFR) was then obtained for each mare by dividing the no. of actual and predicted foals at the 6th reproductive events for the no. of opportunities of doing so (i.e., 6). The predictive ability of coefficients or equations was analyzed by comparing the actuals and the predicted values expressed for each mare using the following statistics:

- the percentage squared bias (PSB; Ali and Schaeffer, 1987), obtained from the formula:
  $PSB=100\ (\mathbf{y} - \hat{\mathbf{y}})'\ (\mathbf{y} - \hat{\mathbf{y}})\ /\ (\mathbf{y}'\ \mathbf{y})$, where $\mathbf{y}$ is a vector of actual and $\hat{\mathbf{y}}$ is a vector of predicted values;
- the mean absolute deviation of residuals (MAD; Vargas et al., 2000) calculated from the formula:
  $\Sigma\ |(y - \hat{y})|/\ n$, where $|y - \hat{y}|$ are absolute differences between actual (y) and predicted values ($\hat{y}$), respectively, and n is the number of observations;
- the standard deviation of residuals obtained from the individual differences between actual (y) and predicted values ($\hat{y}$).

Full dataset and analysis

A full dataset was obtained considering all mares that had at least 3 registered reproductive seasons, born after 1990, located in environmental units with at least 2 observations, and with both known parents. The editing process followed the same rules as for the training dataset, and at the end of editing, LFR was calculated for 3,033 mares' using both coefficients and equations methods. The final dataset consisted of a mixture of actual (n=1,950) and predicted (n=1,443; table 1) LFR both treated as linear variable or arcsin transformed as in Meyer et al (1990) on the basis of suggestion given for proportion variable by Fernandez (1992). For each of 4 different LFR (2 predictive methods and 2 arcsin transformed LFR), a preliminary ANOVA (GLM procedure; SAS Inst. Inc., Cary, NC) was carried out to establish which non genetic effects could be taken into account in the genetic model. Among the non-genetic fixed effects that accounted for a significant part of the total variance were the environmental unit x birth year (EU-BY), and the age 1st foaling (AF). The matrix notation for the final single trait animal model genetic analysis can be written as follows:

$$\mathbf{y}=\mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{e},$$

where **y** is an N x 1 vector of observations, **β** is the vector of systematic fixed effects of order **p** (125 levels for EU-BY and 2 levels for AF; table 1), **u** is the vector of animal effect of order **q** (6,803 animals in pedigree file, i.e. tracing back up to 12th generation for mares with records; table 1), and **e** is the vector of residual effects. Furthermore, **X** and **Z** are the corresponding incidence matrices with the appropriate dimension. The assumptions about the structure of (co)variance were as follows:

$$\mathrm{Var}\begin{vmatrix} a \\ e \end{vmatrix} = \begin{vmatrix} A\sigma_a^2 & 0 \\ 0 & I\sigma_e^2 \end{vmatrix},$$

where σ^2_a is the additive genetic variance, σ^2_e is the residual variance, **A** is the numerator relationship matrix, and **I** is an identity matrix.

Variance components were estimated using the AIREML software from the BLUPF90 family (Misztal, 2008). Heritability values were obtained for normal or transformed LFR (Coefficient and Equations) with the classical formula (Falconer, 1989) and the standard errors of the heritability (SE_{h^2}) were computed applying the following formula of Lynch and Walsh (1998):

$$SE_{h^2}=h^2 \times \left(\frac{Var(\sigma_a^2)}{(\sigma_a^2)^2}+\frac{Var(\sigma_p^2)}{(\sigma_p^2)^2}+\frac{2Cov(\sigma_a^2,\sigma_p^2)}{\sigma_a^2\sigma_p^2}\right)^2,$$

where h^2 is heritability of the trait, σ_a^2 and σ_p^2 are the additive genetic and phenotypic variances of the trait, $Var(\sigma_a^2)$, $Var(\sigma_p^2)$ are their respective predicted error variances, and $Cov(\sigma_a^2,\sigma_p^2)$ is the predicted error (co)variance.

Breeding values obtained for all different LFR expression were estimated for all animals in the pedigree, and annual genetic trends were generated from standardized EBVs considering the mean EBV of recorded mares born in year 2000 and the genetic standard deviation of the trait; The last reference years considered for generating trends accounted for >100 contemporary mares with records or >15 contemporary sires. From this last reference year (i.e., 2007 for mares; 2003 for sires), trends were generated by tracing back the average breeding value of 15 adjacent years. Rank correlations analysis for mares with record (n=3,033) and for stallions that showed a minimum accuracy of 0.60 (i.e., a minimum of 9 recorded daughters; n=86) calculated with the formula reported in Mrode (2005) for EBV from progeny records, were also carried out.

6.3 RESULTS AND DISCUSSION

The comparison between different projection methods based on coefficients or equations is presented in table 2. As expected, all validation coefficients calculated to measure the predictive ability of the two projection methods for incomplete career, showed a progressive reduction, i.e. better fitting, when the timeline for projection was reduced, i.e., from the 3rd to the 5th registered reproductive season. Looking at the average predictive ability, all coefficients resulted lower when projection was carried out by means of the linear regression as respect to the use of ANOVA coefficients (table 2). Therefore, the LFR-E allows the minimum bias (Ali and Shaeffer, 1987) and reduces the residuals between actual and predicted LFR (Vargas et al., 2000). This phenomenon is probably due to the different properties of linear equations as respects to the classification factors, although the difference obtained in this study between the coefficients and equation predictive methods resulted very small, i.e., 0.036 for the percentage square biases, 0.100 lower mean absolute deviation, and a smaller standard deviation of residuals of 0.058. Comparable criteria based on residuals analysis have been previously carried out in comparison of predictive methods or models. For example, Albertsdóttir et al. (2012) used the R method (Reverter at al., 1994) to compare the estimation bias of breeding values for conformation and riding ability in Icelandic horses. Cross-validation methods for analyzing the predictive ability of different models in trotters (Olsen et al., 2012) or, more recently in Spanish Purebred (Pura Raza Español; Sánchez Guerrero et al., 2014) have been also reported. However, both R method and cross-validation have been applied in situations accounting for many thousands of records. Additionally, these methods have been focused on evaluating possible biases in EBVs or predictability of models after splitting randomly the dataset in two parts accounting different percentage of the whole dataset (i.e., training and validation datasets accounting for 75% and 25% of records, respectively). However, in the present study, neither the possible biases on EBVs were an objective of the study, nor the cross-validation could have been easily implemented considering the amount of records available to predict the foal production at the specific endpoint. Therefore, classical coefficients proposed for phenotypes were applied.

Table 3 shows the descriptive statistics obtained on the full dataset, i.e., after the application of the predictive coefficients or equations on foals production of all mares with less than 6 registered reproductive seasons, and after the extrapolation of the LFR in different scenarios accounting also for arcsine transformation of LFR-C or LFR-E. In both cases the transformation of LFR produced an increase in both the mean and standard deviation as compare to the linear LFR-C or LFR-E (table 3). The use of LFR-C produced, both as linear and arcsine transformed variable, a negative index of skewness, i.e., greater density on the left side of the distribution (-0881 and 0.506 for LFR-C and Arcsine LFR-C, respectively). On the other hand, LFR-E and its arcsine transformation determined a longer or fatter right side of the distribution (table 3; figure 1). However, in both cases, the Arcsine transformation of the two LFR values produced a shift toward the right side of the distribution and lower peaks (i.e., lower kurtosis, table3 and figure 1). However, all variables resulted normally distributed as confirmed by all significant coefficients obtained from the univariate procedure of SAS (SAS Inst. Inc., Cary, NC; table 3). Therefore, all subsequent analysis applied have been carried out complying the specific distributional assumptions at the basis the mixed model, although it has been reported that many statistical technique based on normality assumption are more robust that the assumption itself (Shapiro and Wilk, 1965).

The genetic analysis carried out on the 4 different expression of the LFR is reported in table 4. All LFR have shown a detectable genetic variation, although greater for the arcsine transformed LFR as compare to the linear LFR (9.3 on average vs. 4.9, respectively). However, also the residual variance resulted greater for the arcsine transformed variables, leading to almost similar heritability estimates, i.e., 0.25 (table 4). However, the -2log of likelihood resulted lower when the LFR were obtained through the equation based estimates of the foal production at the 6th reproductive season for mares with incomplete reproductive career. Again, as analyzed in the training dataset, this method produced more suitable results. The comparison of results obtained in this study is not easy because of the lack of specific literature on horse. Only the study of Sairanen et al. (2009) have dealt with equine fertility, but the trait analyzed was a foaling rate treated as a dichotomous variable considering the reproductive success of Standardbred and Finnhorse mares repeated over subsequent breeding seasons. Heritability estimates from Sairanen et al. (2009), resulted very low, ranging from 1.1 to 3.0%, indicating that breeding for fertility could not be considered a primary selection goal in the 2 analyzed Finnish breeds. An available comparison of the studied LFR is with the calving rate refereed to lifetime analyzed by Meyer et al. (1990) in beef cattle. Nevertheless, the study of Meyer et al. (1990), reported lower heritability than the values estimated in the present study, ranging from 0.02 to 0.17 depending on the breed considered. However, the variable used in Meyer et al. (1992), was not defined at a specific endpoint as in the present study and the number of opportunities (with maximum value of 11), was accounted in the model as fixed class factor. On the other hand, the main differences with the estimates of Meyer et al. (1990) are in the proportional amount of the residual variance as respect to the additive genetic component. Such changes could be partly due to a general reduction of variability caused by the definition of a specific endpoint for the reproductive career. The raw standard deviation of LFR in the full dataset was indeed about half the value reported on average for cattle and zebu crosses by Meyer et al. (1990), i.e., 0.143 vs. 0.314. Also the phenotypic variance estimated in the present study resulted lower than that reported by Meyer et al. (1990), i.e., 19.69 vs. 69.50, respectively. In spite of such instances, the heritability estimates were much greater than in Meyer et al. (1990), allowing the deduction that genetic determinants of reproductive potential are greater in horse than in cattle or zebu crosses. As in Meyer at al. (1990), the arcsine transformation of the ratios did not produced changes in the heritability estimates, which remained very close to the linear LFR values. In general, moderate-low heritability has been reported for fertility traits in cattle by many authors (Johnston and Bunter, 1996; Robinson et al., 2008; Cammack et al., 2009). However, for traits as age at first calving (Gutierrez et al., 2002), heifer's pregnancy (Evans et al., 1999; Doyle et al., 2000), heifer's puberty (Thallman et al., 1999), and number of calves (Morris et al., 2000), heritability values between 0.20 and 0.30 have also been reported for beef cattle. In general, as pointed out by Cammack et al. (2009), a wide range of heritability values have been reported for the same fertility trait in beef cattle, independently referred to different reproductive season or expressed as lifetime fertility traits. This could be obviously due to the different model implemented, the quality of data and pedigree recording, the amount of available phenotypes and the connectedness of data (Clément et al., 2001), all factors with a recognized effect on the estimates of genetic parameters. In the case of the present study small amounts of data have been used in spite of the fact that all the available information at population level were accounted for. However, the estimated standard errors resulted low (table 4), allowing the deduction that our estimates were not affected by a reduced dataset size. Additionally, the consistency of the genetic parameters and theirs standard errors both as linear and arcsine transformation seem to indicate the absence of possible artefacts due to the distributional properties of the analyzed variables (Meyer et al., 1990). The use of a linear value for LFR or its arcsine transformation did not affect the rank correlation both in groups of mares and in stallions accounting a homogeneous accuracy (table 5) and did not modified the estimates of the genetic trends (figure 2). Genetic trends resulted completely overlapped in mares and with small intersections in stallions' sires of the mares with phenotypic LFR. In general, the observed genetic trend was positive but almost steady in both analyzed categories (figure 2A and 2B), due to the absence of any selection process for fertility in the breed (Mantovani et al., 2005, 2013). However, in spite of general good reproductive performances of the IHDH mares (Mantovani et al., 2005), a small but positive genetic trend for LFR seem promising for a further improvement of the stud-farms' income. Additionally, the use of the LFR could become a tool for management practices addressed to an increase of the fertility rate for breeders, due also to the medium heritability estimated for the trait. The only actual limiting factor that requires cautiousness is the use of EBVs obtained from predicted phenotypes. Indeed, prediction implies reliability lower than 1.0 and, as shown in this study, increasingly uncertain by increasing the timeline of prediction. Therefore, low reliability in the prediction lead to an overestimation in the accuracy of EBVs, with possible negative implications on the selection process. A possible penalization of the accuracy for predicted phenotypes could be then be taken into account, together with a further analysis aimed at investigating genetic correlations obtained by analyzing the actuals and the predicted phenotypes (Cecchinato et al., 2009).

3.4 CONCLUSIONS

In conclusion, the LFR variable calculate at a specific endpoint using actual and estimated no. of foals seem a feasible method to express lifetime reproductive success in IHDH mares. However, the use estimates of foal production at 6th reproductive event through equations performed slightly better than the use of coefficients, and no improvement in the estimates of genetic parameters and EBVs can be obtained by the arcsine transformation of the LFR. A significant genetic variation was detected for LFR, estimating a medium low heritability value. In addition, small but positive genetic trend was observed, although the breed has not yet been selected for LFR. In spite of a good heritability value, a careful use of the EBVs is suggested, because of the prediction leads to an overestimated accuracy for individual with incomplete reproductive career. Last, further analysis on the LFR is required for a final validation of the projection method.

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Table 1. *Characteristics of the training and the full dataset used in the study*

Item	Training dataset	Full dataset
Mares with actual records, no.	1,487	3,033
Mares with projected records, no.	-	1,443
Environmental units x birth year (EU-BY), no.	97	125
Mean records in EU-BY, no.	15.3	24.2
Age at first known reproductive event, mo.	43.7±6.6	43.9±6.5
- 3 years first foaling mares, mo.	36.6±1.8	36.6±1.8
- 4 years first foaling mares, ,mo.	49.1±2.5	49.0±2.4
Animals in the pedigree file, no.	-	6,803
Sires of mares with record, no.	400	602
Dams of mares with record, no.	1011	1,848
Daughters/sire	3.5	4.8
Daughters/dam	1.3	1.5

Table 2. *Predictive ability of coefficients or equations methods used to estimate the foals production at the 6th reproductive season and to obtain the lifetime fertility rate (foal produced divided by the number of opportunities) starting from foals produced after 3, 4, or 5 known reproductive seasons*

Item	Projection method	
	Coefficients	Equations
Projection from 3 known reproductive seasons		
- PSB[1]	0.0186	0.0185
- MAD[2]	0.5878	0.5910
- SDR[3]	0.6923	0.6896
Projection from 4 known reproductive seasons		
- PSB	0.0117	0.0117
- MAD	0.4728	0.4761
- SDR	0.5496	0.5479
Projection from 5 known reproductive seasons		
- PSB	0.0061	0.0055
- MAD	0.2877	0.2820
- SDR	0.3945	0.3756
Average		
- PSB	0.0121	0.0119
- MAD	0.4494	0.4497
- SDR	0.5455	0.5377

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[1] PSB=percentage squared bias, calculated as $100\ (\mathbf{y} - \hat{\mathbf{y}})\ '\ (\mathbf{y} - \hat{\mathbf{y}})\ /\ (\mathbf{y}'\ \mathbf{y})$, where **y** is a vector of actuals and $\hat{\mathbf{y}}$ is a vector of predicted values (Ali and Schaeffer, 1987).

[2] MAD=Mean absolute deviation of residuals, that is □(y - ŷ)/ n, where y and ŷ are actual and predicted values, respectively, and n is the number of observations (Vargas et al., 2000).

[3] SDR=standard deviation of residuals obtained as y – ŷ, where y and ŷ are actual and predicted values, respectively.

Table 3. *Descriptive statistics for lifetime fertility rate (LFR), normality tests (Kolmogorov-Smirnov and Anderson Darling), skewness and kurtosis of the 3,033 data in the full data set obtained combining actual and predicted number of foals after 6th reproductive season and considering different prediction methods for incomplete reproductive career (by coefficients; LFR-C; by equations; LFR-E) and Arcsin transformation of the LFR-C (Arcsin LFR-C) and the LFR-E (Arcsin LFR-E)*

Statistic	LFR-C	LFR-E	Arcsin LFR-C	Arcsin LFR-E
Mean±standard deviation	0.700±0.142	0.699±0.144	0.794±0.195	0.793±0.197
Kolmogorov-Smirnov D	0.16 (P<0.01)	0.14 (P<0.01)	0.15 (P<0.01)	0.11 (P<0.01)
Anderson-Darling A-Sq	82.9 (P<0.01)	78.7 (P<0.01)	67.2 (P<0.01)	60.1 (P<0.01)
Skewness	-0.881	0.144	-0.506	0.197
Kurtosis	0.485	0.986	-0.300	-0.004

Table 4. *Results of genetic analysis carried out on the lifetime fertility rate (LFR) obtained combining actual and predicted number of foals after 6th reproductive season and considering different prediction methods for incomplete reproductive career (by coefficients; LFR-C; by equations; LFR-E) and Arcsin transformation of both the LFR-C (Arcsin LFR-C) and the LFR-E (Arcsin LFR-E)*

Item	LFR-C	LRF-E	Arcsin LFR-C	Arcsin LFR-E
Genetic Variance	4.855	5.016	9.233	9.385
Residual Variance	14.520	14.987	27.765	28.326
Phenotypic Variance	19.375	20.003	36.998	37.711
Heritability	0.251	0.251	0.250	0.249
SE Heritability	0.030	0.030	0.029	0.029
-2log Likelihood	2,776	935	2,720	843

Table 5. *Rank correlation coefficients between standardized EBVs obtained for different expression of lifetime fertility rate (LFR) in Italian Heavy Draught Horse mares with records (n=3,033) or stallions with at a minimum accuracy of 0.60 (n=86). LFR was obtained combining actual and predicted number of foals after 6th reproductive season and considering different prediction methods for incomplete reproductive career (by coefficients; LFR-C; by equations; LFR-E) and Arcsin transformation of the LFR-C (Arcsin LFR-C) and the LFR-E (Arcsin LFR-E)*

Comparison	Mares with actual or predicted LFR	Stallions with≥9 daughters with actual or predicted LFR
LFR-C vs. LFR-E	0.998	0.996
LFR-C vs. Arcsin LFR-C.	0.997	0.995
LFR-E vs. Arcsin LFR-E	0.997	0.995
Arcsin LFR-C vs. Arcsin LFR-E	0.998	0.993

Figure 1. *Class distribution of lifetime fertility rate (LFR) obtained in the full dataset (n=3,033) combining actual and predicted foals after 6th reproductive season; In A distribution of linear LFR obtained by predicting incomplete reproductive career with coefficients (LFR-C) or equations (LFR-E); in B normal distribution of arcsin transformed LFR obtained by predicting LFR with coefficients (Arcsin LFR-C) or equations (Arcsin LFR-E)*

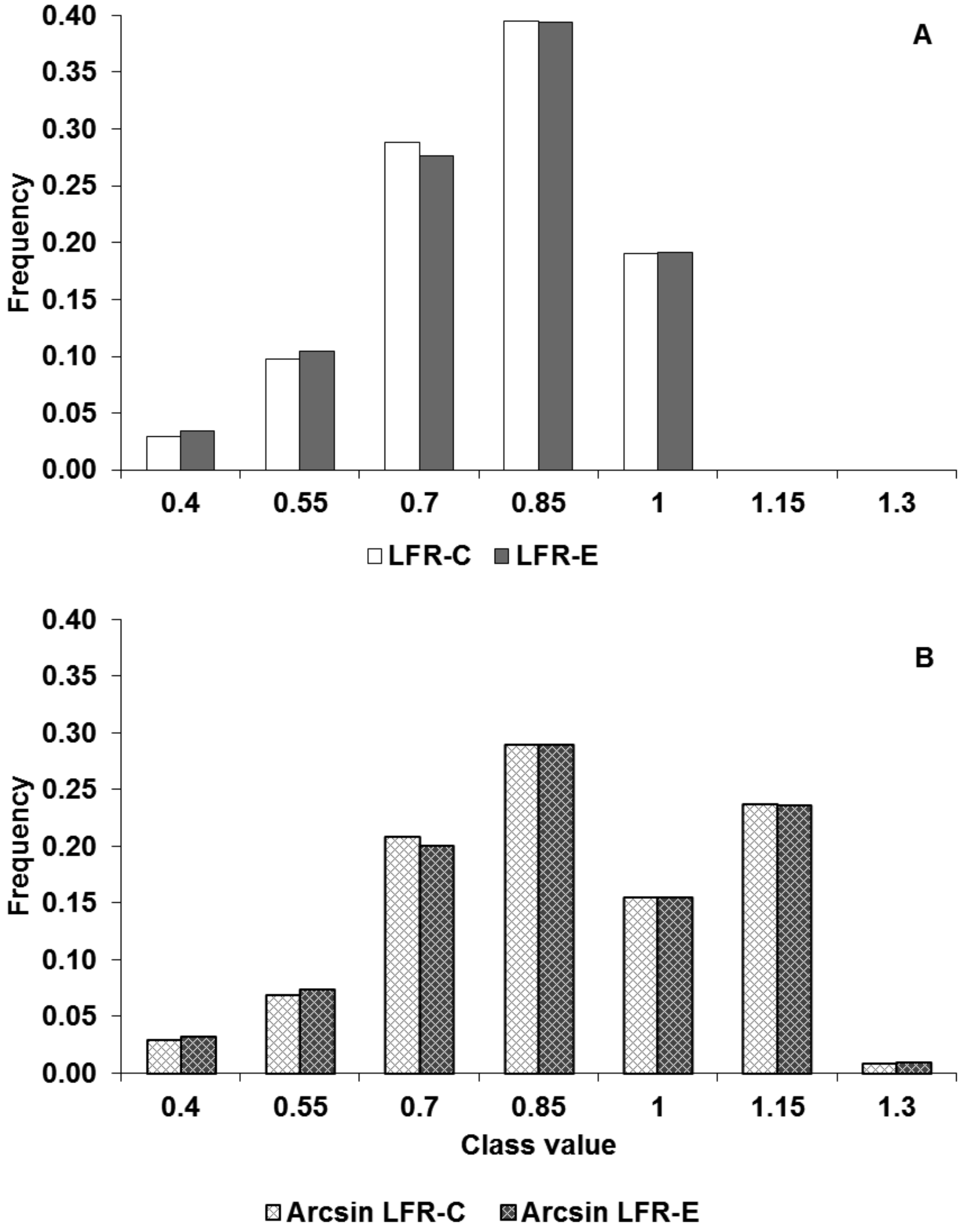
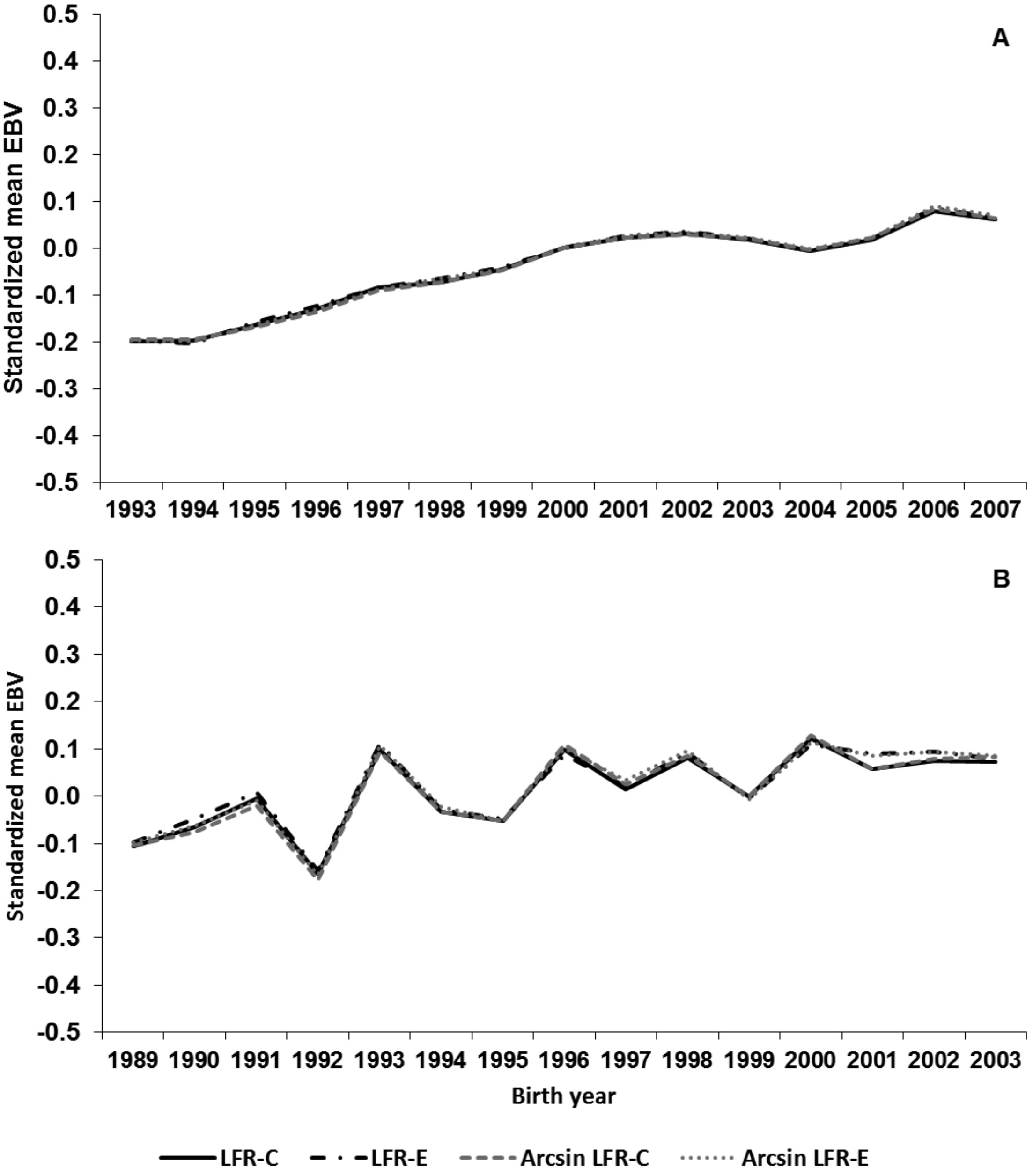


Figure 2. *Genetic trends obtained from mean standardized EBVs by birth year in Italian Heavy Draught Horse mares with record (A; n=2,515) and stallion sires of mares with record (B; n=399). Trends have been obtained for different expression of lifetime fertility rate (LFR), obtained combining actual and predicted number of foals after 6th reproductive season and considering different prediction methods for incomplete reproductive career (by coefficients; LFR-C; by equations; LFR-E) and Arcsin transformation of the LFR-C (Arcsin LFR-C) and the LFR-E (Arcsin LFR-E)*



CHAPTER 7

GENERAL CONCLUSION

This thesis has treated many aspects connected with the present and future of the selection program developed for the Italian Heavy Draught Horse breed and aimed at improving both meat and heavy draught.

The dissertation has been focused also on the description of the history of the breed, the organization of the stud-book, the rules and the criteria for the admission to the stud-book, and the ongoing selection scheme. This description has represented a way for increasing the knowledge on this breed, which remain the only autochthonous heavy coldblood horse type present in Italy. Therefore, the present thesis has represented also a moment for a better knowledge, at international level, of the breed itself.

After many years of a selection process focused on meat and heavy draught through the use of a specific total merit index, chapter 1 has represented a moment of re-analysis of the genetics of the linear type traits scored on young foals, that remain a unique example of early evaluation of the yearly product at population level (i.e., a sort of widespread performance testing). The study conducted in chapter 1 has aimed also at the reanalysis of all non-genetic factors affecting the linear type score, and particularly the validation of a classification system for the stud-farm aimed at accounting in the genetic evaluation also foals located in small stud-farms. Among the grouping methods compared, aimed at retaining the most information as possible in genetic analysis, the most effective solution is based on the combination of housing system, geographical location, and target production, use of vaccination on foals, and mean body condition score of mares. The comparison of models based on the stud-(group) x year x classifier treated as random or fixed effects has indicated the latter as the best fitting solution, and has produced medium heritability values for traits involved in the muscularity development, such as fleshiness, fore and rear diameters, thorax depth and frame size. The genetic correlations among fleshiness, fore and rear diameters, resulted particularly high, ranging from 0.83 to 0.93. Additionally, all traits involved in Total Merit Index (head size, temperament, muscularity, fore diameters, rear diameters) showed a medium-high heritability (from 0.20 to 0.35).

As second step, this thesis has dealt with the genetic analysis of 14 linear type traits scored on IHDH males and females aged 30 months, i.e., at the time of official admission (or exclusion) from the stud-book. This study has represented the first analysis carried out on data recorded at later age than the usual and, in spite of the reduced records available as compared with young foals it has been possible to obtain sound heritability estimates and genetic correlation among analyzed traits. Following the validation method for classifying in group the stud-farms with few observations, the genetic analysis were carried out considering the stud-(group) x year x classifier as main environmental factor. The heritability estimates obtained ranged from h^2=0.03 (upper line length) to h^2=0.40 (frame size). The heritability of the two traits related to outward appearance, i.e., head size expression and temperament resulted h^2=0.31 and h^2=0.21, respectively, and similar estimates were found in traits related to muscularity, i.e., fleshiness, fore and rear diameters, ranging from h^2=0.25 to h^2=0.31. High genetic correlations were obtained among traits related to muscular development: fleshiness and rear diameters (r_g=0.91), fleshiness and fore diameters (r_g=0.52), and fore and rear diameters (r_g=0.76). Positive genetic trends were found in traits of selection interest (muscularity traits, head size and temperament) as effect of indirect selection carried out on a total merit index obtained from EBVs of young foals. The chapter 2 of the thesis indicate that the feasibility of the use of phenotypes obtained later in life as respect to the present selection method base on foals, in light of the good heritability a comparable genetic correlations obtained among traits scored at 30 months of age in IHDH.

In chapter 3, the correlation between the same traits obtained at 6 or 30 months of age were compared, in order to analyse possible substitutions of the early evaluation with phenotypes obtained at adult age. Genetic correlations between traits measured in young and adult age ranged from 0.55 to 0.97, and the phenotypic correlations ranged from 0.15 to 0.51. The lowest genetic correlations between foal and adult scores were observed on thorax depth and fleshiness (i.e., 0.55 and 0.63, respectively). Greater genetic correlations were estimated for the other traits under selection, i.e., fore and rear diameters (0.85 and 0.87, respectively), head-size and expression (0.88) or temperament (0.82). In spite of the good correlation, the analysis of genetic trends attainable with the two alternatives of a direct selection of foals or adults, revealed the superior impact of the selection scheme base on young foals, because of the shorter generation interval.

In chapter 4 the study of a lifetime fertility variable was afforded in order to obtain a management tool useful for breeders in selecting mares on the basis of their reproductive performance. A lifetime foaling rate (LFR) was defined and a validation process of different method for obtaining LFR at a given endpoint of the reproductive career was carried out. Additionally, the study was aimed at analysing the genetic components of the LFR. Results indicated that prediction system resulted well validated when separate linear regression equations accounting for the age at first foaling were used to estimate LFR at 6th reproductive season. The estimated foaling rate showed a moderate but significant genetic variation, and the heritability of the trait resulted medium-low, i.e., 0.25. The arcsine transformation of LFR did not show any improvement of heritability, although increased slightly the fitting of the model. Rank correlations of EBVs for mares with phenotypic records or for sire with ≥9 daughters resulted extremely high comparing the 4 different LFR (average correlation coefficient of 0.996), and, as expected, genetic trends resulted unchanged for the 4 different LFR expression, although almost steady for both mares and stallions. This study has shown the possible implementation of a LFR variable a use for breeding purposes in the IHDH horse.

In general, this thesis has produced worth results that could find implementation within the selection scheme of the IHDH horse; it had confirmed the positive results of selection obtained up to the present time from one side, and it has opened new the prospective based on the maintenance of the present selection scheme but with further prospective for the selection of mares and stallion on the basis of fertility trait in addition to the productive traits already under selection. However, further study aimed at analysing possible alternative in the measurement of fertility, and particularly addressed to the analysis of genetic relationship between fertility and productive trait would be of benefit for selection decision.