

Report to The Irish Draught Horse Society, Ireland

Genetic diversity of the Irish Draught Horse population and preservation of pedigree lines.

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The Food and Agriculture Organisation (FAO) of the United Nations classifies the Irish Draught (ID) horse as an endangered breed due to the declining population size. Falling purebred numbers, combined with a serious threat of genetic erosion, suggest that the ID population is in urgent need of conservation. Genetic diversity is an important component in the consideration of conservation strategies and measures of genetic diversity are becoming widely used in breed management systems. The Irish Draught Horse Society must now identify and preserve its rare bloodlines and explore the genetic resources available to preserve the broadest possible genetic base.

Although Irish Draught Horse pedigrees have been recorded since the turn of the last century, the original records pertaining to the establishment of the early Irish Draught Stud Books were lost in the burning of the Four Courts in 1922 and therefore little is known about either their precise genetic make-up or about the genetic contributions of the founder populations. The loss of authentic records has had profound effects on the lack of pedigree information available and is contributing to the risk of losing rare bloodlines and, unwittingly, condoning dangerous levels of inbreeding. Knowing as much as possible about a population's ancestors (especially the first three generations) can help determine if breeding plans are on track.

The aim of this research was to combine pedigree history and genetic analysis in order to establish the genetic relationships among the Irish Draught population and to assess levels of genetic variation. This will contribute to a more detailed assessment of the risk status of the breed and the establishment of a monitoring and evaluation system. Additionally, this study contributes to the knowledge of the genetic composition of the population and provides the

first molecular characterisation of the Irish Draught horse in relation to other global horse populations. This study will also be valuable in establishing a DNA database to act as benchmark material against which the success of management programmes aimed at the conservation of genetic variability can be assessed in the future.

Introduction

Although natural selection was an important factor in the development of the Irish Draught Horse population, human influence has been the strongest contributor. The focus of horse breeding in Ireland shifted from the production of a multipurpose farm horse to one capable of producing athletic sport-oriented progeny. Given breeders' different ideals, variation in certain characteristics was inevitable. In addition, as a consequence of the frequent lack of in-depth pedigree knowledge coupled with the overuse of a few fashionable stallions with currently "desirable" characteristics, the demise of many old pedigree lines was inevitable. This has resulted in further limiting the breed's gene pool.

The growth of equestrian sports in the latter part of the twentieth century has opened new opportunities for the breed. However, while there is incontrovertible evidence to support the traditional impact of the ID in sport horse production, its modern presence and concentration in the second and third generations of even successful athletes is being progressively diluted. Narrow breeding objectives and intense selection have given rise to concerns over decreases in genetic variation and at the same time the Irish Draught Horse Society (IDHS) is finding it increasingly difficult to maintain sufficient population numbers to ensure its long-term survival.

Genetic diversity refers to the variation of individual genes, and provides a mechanism for populations to adapt to their ever-changing environment. Loss of genetic diversity erodes our capacity to maintain and enhance ID production and decreases the ability to respond to changing social or economic conditions. The genetic diversity of a species is always likely to accommodate change. No matter how many variants of a gene are present in a population today, only the variants that survive in the next generation can contribute to the diversity of the species in the future. Once gene variants are lost, they cannot be recovered. This genetic erosion is the loss of genetic material that occurs as a result of specific selection. For example, the selection for lighter boned, taller horses (often of specific bloodlines) which nudge the ID

breed away from the traditional breed type may cause genetic erosion within the breed. The most extreme example of genetic erosion is extinction. The genetic characterization of the ID is the first step in assessing its sustainable management or conservation.

Mitochondrial DNA (mtDNA) has been used extensively by scientists in the last three decades as a tool for inferring the evolutionary past of populations. This tool has proven invaluable in genetic diversity studies. MtDNA, present in every cell of every animal's body, is inherited solely from the mother and undergoes no recombination with paternal DNA. Females always pass on their mtDNA as an intact package. Males on the other hand never pass on their mtDNA. Thus, all progeny from a single mare share identical mtDNA sequence. In addition, the dam's maternal relatives also share this same mtDNA. Therefore, mtDNA makes possible the reconstruction of dam line relationships, without influence from the sires used over the generations. **Figure 1.** illustrates mitochondrial DNA inheritance.

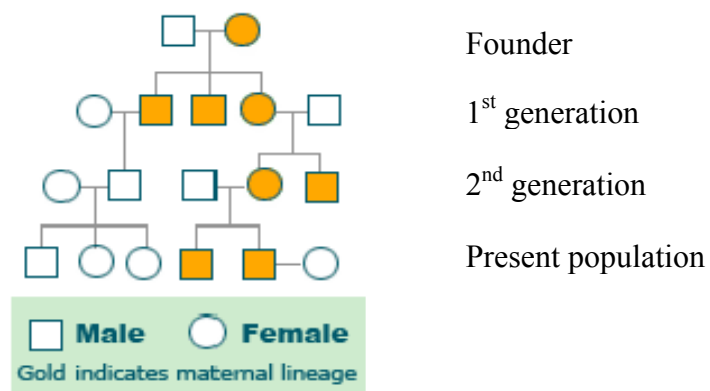


Figure 1: Mitochondrial DNA (mtDNA) inheritance from mother (circle) to offspring. Note that even though the male (square) inherits mtDNA from his mother, he cannot further contribute to his future progeny.

MtDNA is inherited as a single unit and does not change from generation to generation except via mutation. MtDNA accrues mutations rapidly and is highly variable. The control region is the most variable portion of the mammalian mtDNA genome, making it useful for studies of genetic variation among domestic animal populations. MtDNA sequences either match or they do not. Assessing the similarity / dissimilarity of the sequences can indicate the extent of relationship between individuals. The combination of mtDNA research with recorded pedigrees provides a powerful approach to understanding female relationships.

In this study, genetic diversity in the ID has been investigated by combining pedigree analysis and variations in mtDNA sequence enabling inference about population history and the processes that have led to existing genetic variation. This has also contributed towards a greater understanding of the contribution of original ID pedigrees.

Results

Sample Selection

In February 2004, the Irish Horse Register (I.H.R.) contained 210,542 records. Through maternal pedigree analysis, from an 878 male reference population (i.e. total number of male progeny born in the period 2000-2003 with R.I.D. and I.D. mares), 414 founder females were identified. The definition of a ‘founder’ animal in scientific terms is directly related to information available in pedigree records. If the dam of an animal is not recorded in a pedigree then for the purposes of this analysis that animal is considered to be a ‘founder’ mare. A total of 120 founder females were selected recording at least three generations which could be traced back to the 1960s. From these founders, fifty-nine male progeny were randomly selected to represent the ID founder mares in Ireland. **Figure 2** shows the 59 founder ID mares selected with their year of birth and the number of recorded male descendents born 2000–2003.

In order to compare diversity in the founder population with existing genetic diversity a ‘modern’ population containing 286 individuals was created by computer simulation using pedigree and genetic information from the founders to infer current variation.

DNA Extraction, Amplification, and Sequencing

DNA was isolated from hair-root samples of 59 Irish Draught horses and mtDNA was amplified and sequenced. These sequences were subsequently deposited in GenBank (GenBank is an annotated collection of all publicly available DNA sequences. It is the largest worldwide genetic database collecting all known DNA sequences).

Number of males born 2000-2003 in each founder ID mares pedigree

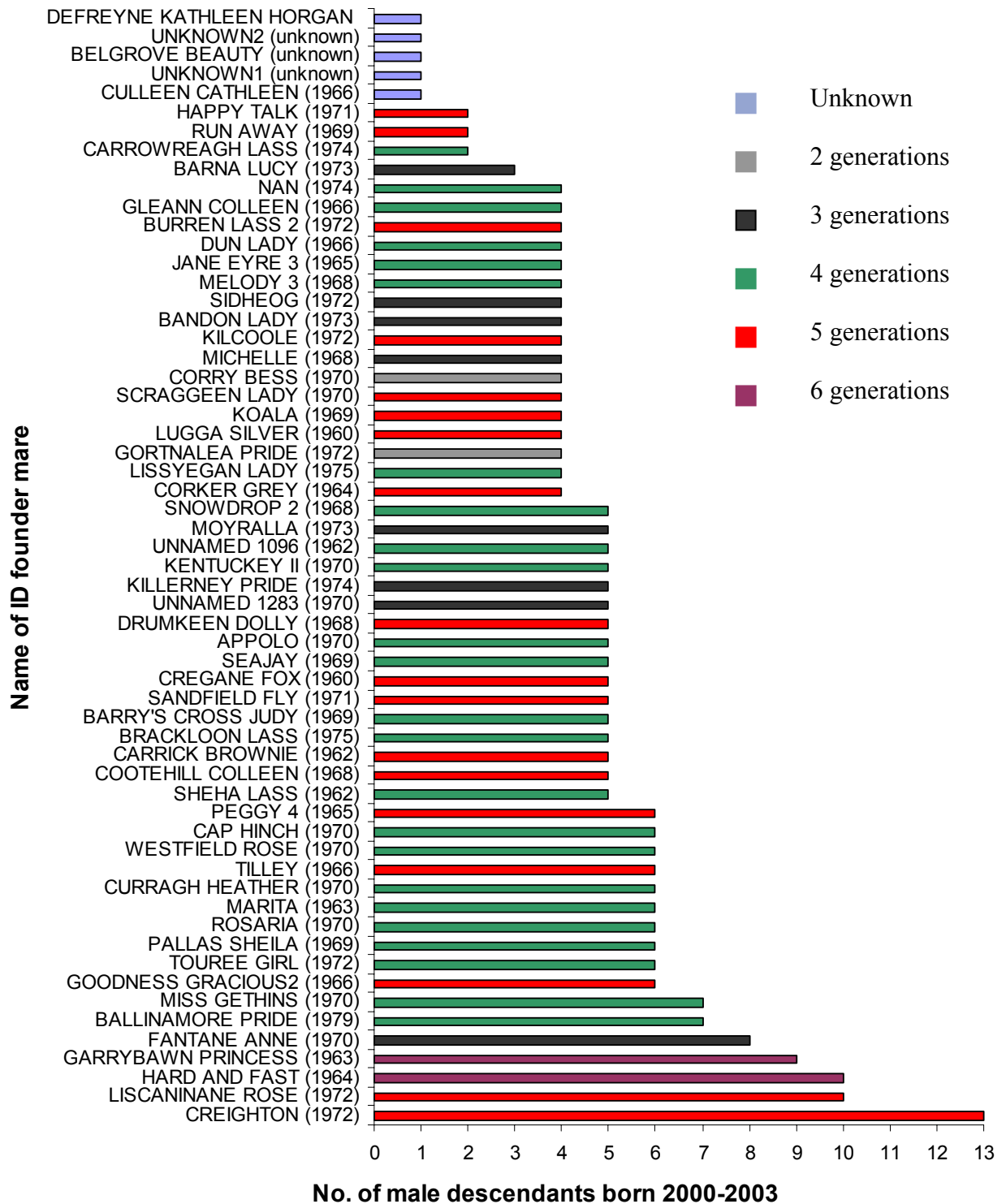


Figure 2: Irish Draught founders selected (with year of birth in brackets) illustrating the number of male descendents in the population from 2000-2003. Bar chart is colour-coded illustrating the number of generations identified from pedigree records.

Maternal lineages

Genetic analysis identified 35 distinct maternal lineages in the 59 founder Irish Draught sequences dating back to the 1960s. In the modern population only 25 maternal lineages remained (2005). This is a 28% loss of maternal lineages.

DNA sequence No.	Founder Names
Sequence 1	JANE EYRE 3, KENTUCKEY II, NAN, SHEHA LASS, CORKER GREY, GOODNESS GRACIOUS 2, HAPPY TALK, BANDON LADY, CREGANE FOX, HARD AND FAST
Sequence 2	UNNAMED 1283, TOUREE GIRL, CREIGHTON, BRACKLOON LASS, CULLEEN CATHLEEN
Sequence 3	MELODY 3, CARRICK BROWNIE, BALLINAMORE PRIDE, CORRY BESS
Sequence 4	KILLERNEY PRIDE, BURREN LASS 2, KOALA
Sequence 5	CARROWREAGH LASS, GORTNALEA PRIDE, UNKNOWN2
Sequence 6	RUN AWAY, LISSYEGAN LADY, DEFREYNE KATHLEEN HORGAN
Sequence 7	CAP HINCH, GLEANN COLLEEN
Sequence 8	MICHELLE, LISCANINANE ROSE
Sequence 9	SEAJAY
Sequence 10	APPOLO
Sequence 11	DUN LADY
Sequence 12	DRUMKEEN DOLLY
Sequence 13	MISS GETHINS
Sequence 14	FANTANE ANNE
Sequence 15	UNNAMED 1096
Sequence 16	MOYRALLA
Sequence 17	PEGGY 4
Sequence 18	SNOWDROP 2
Sequence 19	COOTEHILL COLLEEN
Sequence 20	UNKNOWN1
Sequence 21	BELGROVE BEAUTY
Sequence 22	PALLAS SHEILA
Sequence 23	LUGGA SILVER
Sequence 24	SCRAGGEEN LADY
Sequence 25	ROSARIA
Sequence 26	BARRY'S CROSS JUDY
Sequence 27	SANDFIELD FLY
Sequence 28	MARITA
Sequence 29	BARNA LUCY
Sequence 30	KILCOOLE
Sequence 31	CURRAGH HEATHER
Sequence 32	TILLEY
Sequence 33	GARRYBAWN PRINCESS
Sequence 34	WESTFIELD ROSE
Sequence 35	SIDHEOG

Table 1: 59 founder females displayed into the 35 maternal DNA sequence groupings. Each DNA sequence originates from a common female ancestor. The majority (55%) of ID dam lines stem from eight maternal sources.

The degree of similarity, or probability of identity (PI), calculates the probability that two individuals in a population share an identical sequence. This statistic was calculated for the ID

founders (1960s) and the simulated modern ID population (2005). The PI for the founders was 0.0583 and the PI for the ‘modern’ population was 0.0751.

Table 1 shows the sharing of maternal lineage sequences. It reveals how ID mares formerly thought to stem from unrelated mares, may have common female founders. Additionally we have found that 55 % (32 founder females) of the founder ID population originated from eight common maternal lineages (DNA sequences 1-8).

Irish Draught mtDNA sequence sharing with other horse populations

The genetic relationships between ID and other populations either documented or alleged to have contributed to the formation of the Irish Draught population (Connemara, Kerry Bog Pony, Clydesdale, Cleveland Bay, Shire, Thoroughbred, Scottish Highland, Exmoor, Anatolian, Arab, Orlov plus 5 breeds from Spain and Portugal: Andalusian, Sorraia, Asturcon, Lusitano, CabalodeCorro) were investigated. The highest proportion of mtDNA sequence sharing was with Caballo de Corro (93%) closely followed by the Andalusian (83%). **Table 2** illustrates the percentage of sequence sharing among 16 horse populations that may have contributed to the formation of the Irish Draught population.

Population	% Sharing
Irish Draught	100
Caballo deCorro	93
Andalusian	83
Clydesdale	80
Scottish Highland	75
Orlov	72
TB	68
Connemara	67
Exmoor	67
Kerry Bog Pony	67
Asturcon	62
Anatolian	60
Sorraia	60
Cleveland Bay	60
Shire	50
Lusitano	38
Arab	35

Table 2: Mitochondrial DNA sharing frequency (%) among selected breeds with the Irish Draught population.

Discussion

This study lays a foundation to understand the genetic contributions to the endangered ID population and provides a reference to monitor maternal lineage preservation in attempts to prevent further genetic erosion. These data provide an important reference for the consideration of future breeding and population management practices aimed towards maintaining population genetic diversity.

One of the factors favouring the preservation of genetic diversity from ‘founder’ animals is the extent to which the contributions of the various ‘founders’ are balanced or equal. The ID reference population (2000-2003) has been identified in this study as comprising 414 founder females, while a previous study (Helen O’Toole, UCD MAgSc Thesis 2001) estimated the ‘effective number of founders’ as 135. The ratio of the ‘effective number of founders’ to the ‘actual number of founders’ indicates the balance of the contributions made by the ‘founder’ animals to the population. The discrepancy between the ‘actual number of founders’ (414) and the ‘effective number of founders’ (135) indicates that not all founders have had an equal contribution to the reference population. This suggests a loss of genetic diversity due to the preference for popular/fashionable lines.

Coupling genetic investigations with pedigree studies can add significant value to understanding and assessing risk status. It was demonstrated that of the 59 founder Irish Draught female lineages the mare “Creighton” (1972) has made the greatest mtDNA contribution to the modern day male population (born 2000-2003), followed closely by “Liscaninane Rose” (1972) and “Hard and Fast” (1964).

Thirty-five distinct mitochondrial lineages were found among the 59 founders (**Table 1**), indicating that no fewer than 35 females have contributed to the formation of the Irish Draught horse population. Interestingly eight of these 35 lineages contained 32 of the 59 founder females. This suggests that nearly 55 % of the founder population had eight common maternal lineages. It is not possible from these data to establish how recent the common ancestors might have been. They could equally be within one generation or thousands of years ago. By comparison, a recent similar study found that as few as 12 founders may have contributed to the formation of the thoroughbred. This suggests that present day ID mtDNA sequences derive from a relatively diverse foundation stock.

In a wider comparison the ID founders were found to share mtDNA sequences far less often than has been observed in other horse populations. These data show that the possibility of a mtDNA match in the Irish Draught was 0.058. This means that of 100 randomly chosen Irish Draught animals, no more than six are likely to share identical sequences stemming from common maternal origins. However, **Table 1** illustrates the reality showing that some animals shared common ancestors more than others. Sequence sharing among the ID founders was three times less than that observed in the thoroughbred (0.15) and is similar to estimates in the Arab horse (0.05). Therefore, despite concerns about loss of genetic variation in the ID, considerable diversity was detected in the founder ID mtDNA sequences. The ID stem from diverse original stock.

Despite considerable diversity among the founder population (1960s), comparisons with a 'modern' population suggest that there has been a substantial loss (28%) of maternal lineages over a relatively short time period (45 years). Nevertheless, even with this significant recent loss in diversity the ID may not be in immediate danger from genetic erosion while the population maintains comparable diversity levels with other horse populations. Importantly however, current conservation efforts must be monitored and maintained to prevent further loss of genetic diversity. Future management strategies might include genetic analyses to assess continued maintenance of diversity, which is crucial for the population to survive in the long term.

To provide wider inference about ID history the ID population was examined in a global context by studying 962 sequences from 72 distinct horse populations. Like most horse populations, the ID shares mtDNA sequences with other populations despite geographic distance. Historical documentation has suggested a Spanish origin for many of the founders of the Irish Draught. Examining the contributions from 16 populations this study found the greatest proportion of ID sequence sharing with the Spanish population, Caballo de Corro (93%) closely followed by the Andalusian (83%). However, numerous breeds have contributed to the ID history and it is therefore not surprising to find a broad genetic base for the ID population. The English Clydesdale shares 80% and the Russian Orlov 72%. This high percentage in the Russian Orlov horse was not entirely surprising as this was the horse of choice imported to England to improve the quality of the thoroughbred. Unfortunately, no mtDNA sequence from any French horse populations has been deposited in the GenBank

database preventing any comparison with French breeds. The lack of any clear pattern of sequence sharing with other populations cannot allow us to state with any accuracy the exact origin of the ID. This is principally a reflection of modern horse husbandry and the high mobility of the horse which has resulted in a great deal of mixing of gene pools. The ID might equally descend from a single diverse source population as it may have evolved from several populations, though historical records suggest the latter. The submission of ID mtDNA sequences to the GenBank database for the first time will contribute to a wider understanding of domestic horse origins and will aid future genetic diversity studies.

Mitochondrial DNA studies illustrate only one aspect of inheritance, namely the female contribution. Future studies will be necessary to clearly discriminate between populations. In particular it has been shown that microsatellites contain information with great power to understand detailed population structure, history and relationships. More detailed genetic information about the Irish Draught horse may be important in the consideration of reopening the Irish Horse Board studbook to outside bloodlines while avoiding distorting their fundamental gene pool.

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Publications

A more detailed account of the project will be published in the scientific literature (2006). For more details please contact Emmeline.Hill@ucd.ie.