

## WILDLIFE ECOLOGY

### **A review and criticism of the research article “Mitochondrial DNA sequence diversity in extant Irish horse populations and in ancient horses”**

**by Odhrán Smith, May 2009.**

Research article: *Mitochondrial DNA sequence diversity in extant Irish horse populations and in ancient horses*, A. M. McGahern et al, 2006. Published in *Animal Genetics* 37, 498-502.

This particular research paper focusses on the equine mitochondrial DNA (mtDNA) sequence variation in the three native horse populations – Connemara Pony, Kerry Bog Pony and the Irish Draft Horse. Sixty-nine other horse populations were investigated for contextual purposes. In addition, ancient DNA sequences were obtained from three archaeological equine specimens.

The article mentions the most important reasons for research: 1) despite breeding records being in existence for over 100 years, little is known about the genetic make-up or contributions of the indigenous Irish equine breeds (Connemara Pony (CON), Kerry Bog Pony (KB) and the Irish Draft Horse (ID)); 2) due to the decline in agricultural uses (including hunting, carriage transport, peat harvesting) the presence of the ID horse is being diluted and the sire lineage is becoming very limited, and there are only 94 approved ID stallions and approx. 900 ID mares (Slavin 2005); 3) the Kerry Bog Pony faced imminent extinction due to modernisation in farming, removing its traditional roles (carry peat for domestic consumption, delivering coal, transport); 4) the Connemara Pony has seen a sharp rise in the number of ponies registered in the Connemara Pony Breeders Society Stud Book (in Vol. 1 in 1926 there were 9 stallions and 26 mares listed; in Vol. 21 1999 there were 1043 stallions and 11,621 mares detailed). From my own point of view, I was particularly interested in the genetic background of the KB in relation to the other indigenous breeds, due to historical evidence suggesting that the Kerry Bog Pony (formerly referred to as Irish Hobbies) may have originated from northern Spain (Asturia), which is home to the native Asturcón breed.

The study looked at the mtDNA diversity of the KB, ID and CON populations and placed them within context of 69 other horse breeds. I think this was a good method to use for eliciting the demographics and evolutionary path of closely related species. Three ancient Irish horses were sequenced to help draw conclusions about the present diversity found in the above breeds. Fifty-nine male descendants were selected at

random from 120 founders of the ID population. Thirty-three Kerry Bog Ponies were selected based on phenotypic traits and as there was no pedigree history for this breed, individuals were DNA-typed using StockMarks for Horses Equine Genotyping System to determine relatedness. A further 6 KB ponies were included due to the fact that no parent-offspring pairs were found in the original sample of 33. Twelve CON animals were included that have been described in a previous study (Hill *et al.* 2002). These were selected to represent the CON founder female families (AF481247 AF481258).

Four samples from ancient Irish horse remains were obtained however only three were used, as the sample from Lough Shad, Co. Roscommon did not generate amplifiable DNA. The other three samples were 1) a metatarsus from Edenvale, Co. Clare, 1595 ± 78 cal BP (calibrated, years Before Present); 2) a tooth from Kesh, Co. Sligo, 1472 ± 56 cal BP; 3) a scapula from Shandon, Co. Waterford, 32,255 ± 712 cal BP. A sample from Derbyshire, England was also included.

Genomic DNA was isolated from 39 KB ponies and 59 ID horses and subsequently the mtDNA was amplified. DNA extractions from the ancient material was performed at Trinity College Dublin from either bone or tooth samples. Samples were validated by an independent laboratory in Cambridge. Amplicons were sequenced directly and a representative sampling of PCR (Polymerase Chain Reaction) resultants were then cloned. They then made a consensus sequence which reached greater than 95% confidence levels at all bases. McGahern *et al.* deposited the sequences in GenBank (ID: DQ327891 DQ327949; KB: DQ327852 DQ327890; Ancient samples: DQ327848 DQ327851). Eleven haplotypes were found in the 12 CON founders; 28 distinct maternal lineages were found in the 59 founder ID sequences and 17 haplotypes were identified in the 39 KB pony founder female lineages. The team then went on to calculate the probability of two individuals in a population sharing an identical haplotype (referred to as PI, probability of identity). This was estimated at 0.097 for CON, 0.058 for ID and 0.0900 for KB. Investigating sequence sharing led to the conclusion that ID mtDNA derived from a widespread foundation stock, while CON and KB matrilineages were more restricted.

Using nomenclature from Jansen *et al.* (2002), they grouped the sampled breeds into haplogroups. Haplogroups A, B, C and D are the most common in European horse sequences. Of the Irish Draft horses, 48% belonged to haplogroup D. The remaining ID sequences were grouped into haplogroup A (32%), C (12%) and B (8%). The sequences from the Connemara pony were grouped into haplogroups D, A, B and C (42%, 25%, 17% and 17%, respectively). I was most interested by their findings that the Kerry Bog Pony had an unusual distribution of sequences: the majority (31%) were assigned to haplogroup E, and the remainder to haplogroups C and A (26% and 23%, respectively).

The study found that all the ancient Irish horse remains studied were assigned to haplogroup A. Using Fisher exact tests, a high level of clustering was found when the ancient Irish horse remains were compared with modern Irish ( $P = 0.026$ ) and modern British ( $P = 0.020$ ) populations. It was therefore concluded that ancient sequences did not correlate to the distribution of the modern Irish horse populations. Even though the study referred to a single ancient British sample found in haplotype D, in my opinion they gave a balanced view on this issue by indicating that this may not be taken as conclusive evidence of single haplotypes present in ancient populations: other studies have indicated that a more varied spread of haplotypes may exist in ancient horse samples, citing studies from Vila *et al.* and Keyser-Tracqui *et al.*

The study cautions that it is not as easy to observe geographic patterns of domestication in horses, as it is in other large breeds. However they do point out documentation from Fell 1991 and O'Toole 2001 suggesting a Spanish origin for many of the founders of the Irish Draft Horse, which according to Lopes *et al.* 2005 could be a potential origin for haplogroup D. Unfortunately, one of the most interesting conclusions of the study was inadequately researched in my opinion. They allude to the haplotype percentages of the Kerry Bog Pony. It has only 13% of haplotype D, which is a common European haplotype (35%). Haplotype E was represented far above that of the other indigenous breeds at 31%. They point out that the KB pony has the highest percentage of Haplotype E investigated in any breed to date. Sixty-six per cent of this rare haplotype is represented in the British Isles: twelve KB ponies, 6 Shetlands and one Thoroughbred.

The DNA molecule is robust and stable and as such is useful for forensic investigations such as the determination of purity of animal breeds (IVJ, Vol. 55). Bearing this in mind, I think the team were well justified in using it for their study of the ancient Irish horse population. I think this study was quite thorough in its approach.

As an aside, in 1994 Wetherbys Ireland DNA laboratory commenced blood typing the Kerry Bog pony as part of an effort to define the breed due to efforts by a local conservationist, John Mulvihill. The laboratory investigated 21 genetic sites for the transmission of 127 identified alleles using Allo-immuno Red Cell typing, Biochemical Protein Polymorphism and DNA Dinucleotide Microsatellite fragment analysis. They found up to 99% sharing in the frequency data of the alleles but important frequency differences between the ID, CON, Sporthorse and Thoroughbred horse populations. They mention "one particular marker is either absent or extremely rare in the other 4 breeds but is present in 8% of the Kerry bog population". They also demonstrate a genetic distance matrix of 35% against Thoroughbreds, but only 5% against Connemara ponies.

It is evident from the above studies that the KB pony shows some atypical genetic characteristics for the region in which it is found (However, horses are very mobile animals, and this gene migration contributes to a lack of phylogeographic structure (Tatjana Kavar *et al.*, 2008). Is it also noteworthy that no wild horses remained in Ireland after the last Ice age due to extinction and the earliest evidence of domesticated horses in Ireland is from 4400 years before present (McGahern *et al.* 2006). Historically, it is thought that military and trading links between Spain, Portugal and Ireland may have brought some of a small race of ponies, the Celdones, to Ireland\*. Isaac Ware visited Co. Kerry in 1720 and observed that Kerry Bog Ponies were formerly called Asturiones. Irish horses were frequently used during the Peninsular wars of 1804-1814 and during WWI. Looking at the breed standard, there are many similarities between the Asturian breed (Asturcón) and the Kerry Bog pony (in terms of height, typical colouring, build, temperament etc.). Also, it is known that the Asturcón were a comfortable ride due to their ambling gait, and this made them popular as a ladies' mount.

They were called *haubini* in France, and the etymology of this word suggested that it later became *hobbye* and eventually *hobby horse*. This would contradict the common perception in Ireland that the word Hobby Horse came from the practice in Irish-speaking regions of saying "Hup, Hup" to call the horse home during *obaireacht* ('working').\*\* Another explanation is that a light cavalry originating in Ireland in the 13<sup>th</sup> century were referred to as Hobelars. They rode Hobbies, and in this sense the word is said to derive from *hobin* a French word thought to originate from the Irish *obann*, meaning 'swift'. Unfortunately, I cannot find any scientific publications indicating the phylogenetics of the Asturcón to support the link suggested above. Also, the morphological likeness and degree of relationship between these pony breeds is not necessarily an indication of true evolutionary history of the populations (J. Jordana *et al.*, 1995). It would be especially interesting to learn the haplotype group distribution of the Asturcón for comparative reasons to lend scientific credence to historical evidence.

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