

# Comparative genetic characterisation of the Kerry Bog Pony breed using a panel of high-density genome wide markers.

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## Project Overview:

This project endeavoured to perform an in depth comparative genetic characterisation study for the Kerry Bog Pony (KBP) breed. 192 breed representative samples (26 Males and 166 Females) were selected based on breeding activity status. This project utilised recent advancements in molecular technology which allowed for analysis of ~670,000 genetic sites across each Kerry Bog Pony genome.

Generation of such extensive genotype profiles per pony, provided suitable data to accurately assess levels of genomic inbreeding, relationships and breed purity that exists within the breed. To provide further information, compatible genotype datasets from 39 other global Equine breeds (Petersen et al 2013 and Winton/Hegarty et al 2013) were also merged with the Kerry Bog Pony dataset to allow for comparative analysis. Furthermore, all 192 Kerry Bog Ponies underwent genotyping for a range of coat colour markers to establish frequencies of core coat genes within the Kerry Bog Pony breed.

This study provides insight into the current population wide genetic status of the Kerry Bog Pony breed and has established genetic metrics usable for future breed management conservation strategies.

## Genotyping and Genomic Inbreeding:

Archived hair samples for 192 KBP animals underwent DNA extraction and subsequent genotyping on the Affymetrix Equine 670K SNP array. 190 (25 Males and 165 Females) out of 192 samples passed genotyping QC/Sex checks and these 190 samples were used for further analysis purposes.

Genomic inbreeding percentages ("F" Inbreeding Co-Efficient X 100) were established for the KBP at both an individual animal and breed average level, where higher inbreeding percentages correlate to higher inbreeding levels. Inbreeding ranged from 0 - 26.5% for individual KBP animals and a breed average across all 190 KBP animals was estimated at 3.4%. The majority (157 of 190 i.e. 83%) of animals had an inbreeding percentage of ≤5% and 8 animals were identified as having an inbreeding percentage of >10% - see Figure 1 for complete distribution breakdown. Comparative inbreeding analysis against 39 other global Equine breeds to the Kerry Bog Pony breed average of 3.4%, revealed that the KBP has an average inbreeding level towards the lower end of the scale, in comparison to other Equine breeds (Figure 2).

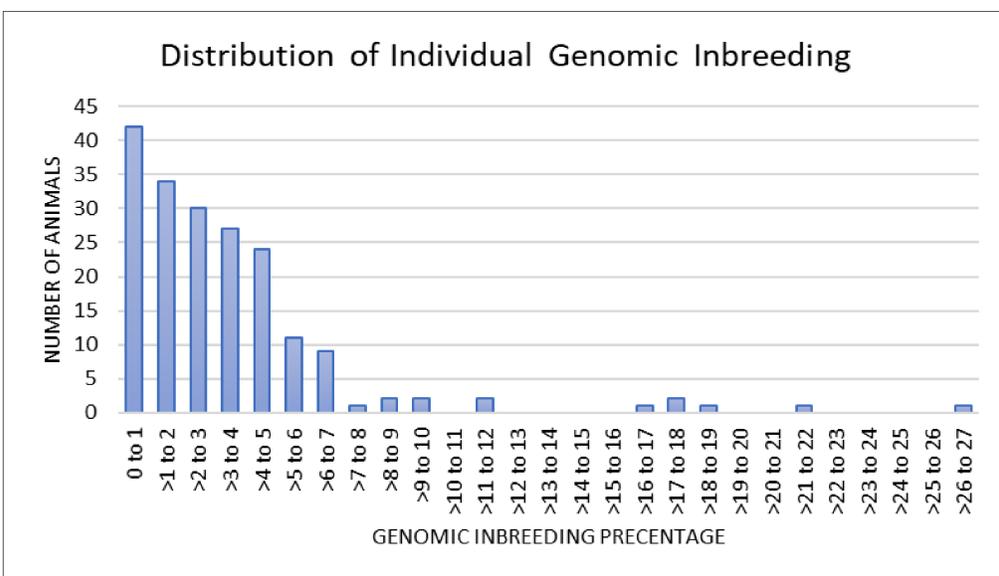


Figure 1: Number of animals within each genomic inbreeding percentile, ranging from 0 to 27% for all 190 KBP animals.

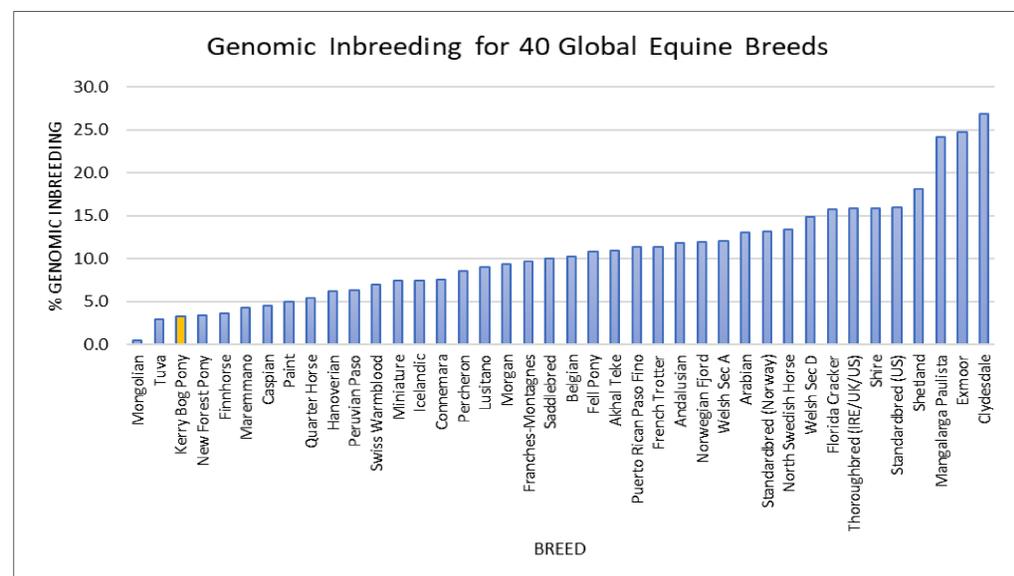


Figure 2: Average genomic inbreeding across 40 breeds \*, with KBP highlighted towards the lower end of the scale in comparison to other Equine breeds.

## Genomic Relationships and Breed Purity:

Genomic relationship percentages were established for all mating combinations of 25 Males and 165 Females i.e. 4,125 possible matings. Genomic relationship percentages and their likely indicative relationship include, 0%=Unrelated, 3.125%=Second Cousins, 12.5%=First Cousins, 25%=Half Sibling or Grand Parent-Grand Child and 50%=Full Siblings or Parent-Offspring, and all 4,125 relationship values were categorised into incremental groups of 6.25% to establish the number of possible matings within each relationship category (Figure 3). The majority of mating combinations (3,918 out of 4,125 i.e. 95%) had a genomic relationship value of ≤12.5% i.e. likely to be First Cousin relations or less, and 67 matings combinations had a genomic relationship value of >25% i.e. likely to be Half Sibling relations or more.

Genomic breed purity was also evaluated via comparing all 190 KBP genotypes to genotypes from 8 Equine breeds of similar pony type stature or close geographical proximity – Welsh Section A and D, Shetland, Exmoor, Connemara, Icelandic, New Forest and Fell pony. Allocation of KBP purity percentages was based on screening all KBP samples for fixed genetic signatures associated with each of the 8 comparative breeds and post screening, unallocated breed purity was deemed representative of a hypothesised KBP signature. Such breed purity results may be subject to change depending on comparative breeds and analysis models used, however breed purity percentages were considered from the most statistically likely model to best represent population substructure between analysed breeds. Subsequent breed purity percentages were assessed for both 165 Female and 25 Male subgroups, and a higher average KBP breed purity was observed within the Males at 76%, in comparison to the Females at 34%. In general outcrossing was also observed within both Male and Female subgroups and outcross breeds (of >10% composition) in order of average prevalence were – Welsh Section A, Connemara, Shetland and New Forest Pony (Figure 4).

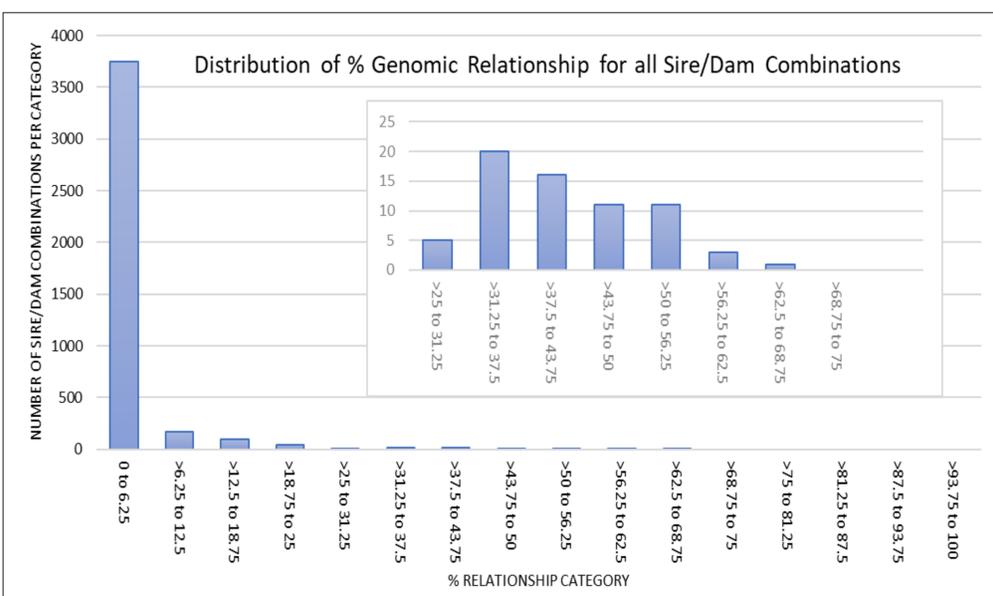


Figure 3: Number of KBP Sire/Dam combinations per Genomic Relationship category. Expanded view of categories between 25 and 75% also shown.

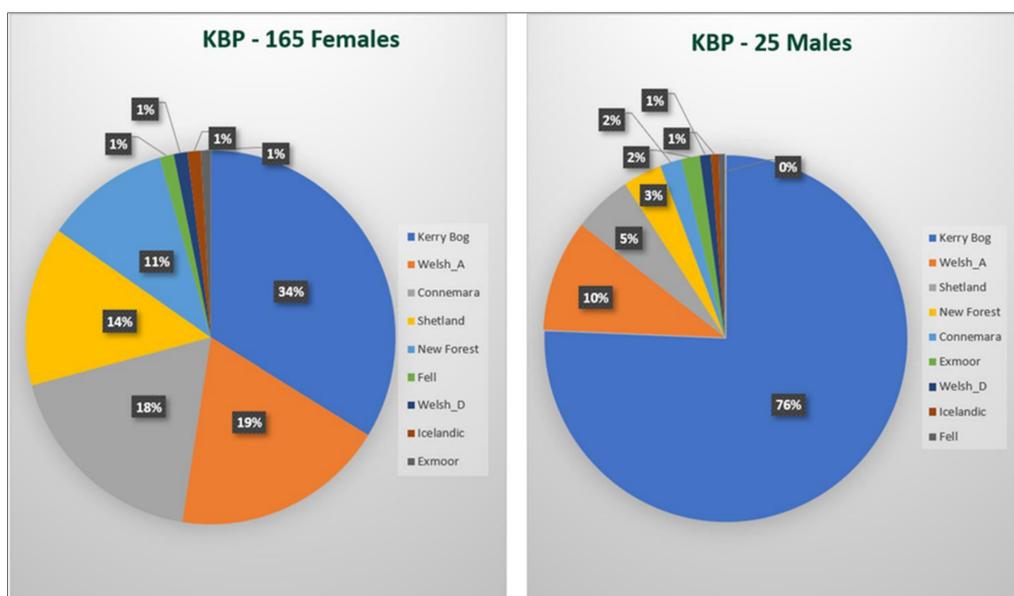


Figure 4: Average percentage breed composition for 165 KBP Females and 25 KBP Males, with a hypothesised KBP signatures screened against 8 breeds of similar type stature or close geographical proximity.

## Coat Colour:

All 190 KBP samples underwent genetic based coat colour analysis, whereby individual genotypes were established for Red/Black Extension, Agouti Distribution, Tobiano Spotting pattern and Cremello Cream genes (Figure 5). Genotyping for Recessive Red "e" and Dominant Black "E" alleles revealed that based on this representative group of 190 KBP animals – 37.89% of KBP's have chestnut base coat colour. It was also noted that 15 out of 190 Red/Black Extension genotypes conflicted with recorded coat colour phenotypes e.g. Genotyping "ee – Chestnut" and having a recorded Bay/Brown coat colour. The Agouti Dominant "A" allele is known to restrict black colour to the points, while Agouti Recessive "a" distributes black colour evenly and Agouti genotype percentages were "AA" = 29.47%, Aa = 52.11% and aa = 18.42% across 190 KBP samples. Considering this near even spread of "A" and "a" allele frequencies and that the Agouti gene controls the distribution of black pigment, which is present within the KBP at an average of "Ee" = 43.68% and "EE" = 17.89%, a range of Bay, Brown or Black coat colours are also to be expected within KBP animals - explaining the wide coat colour spectrum observed across the breed.

All 190 KBP samples underwent Tobiano Spotting pattern genotyping whereby due to the dominant nature of the trait, animals that are Tobiano Carrier (N/TO) and Tobiano Homozygous (TO/TO) will display spotting patterns. No Tobiano homozygotes genotypes were observed within this representative group of 190 KBP samples. A Tobiano Carrier percentage of 3.16% was observed i.e. animals display spotting coat colour, however a Non-Carrier genotype percentage of 96.84% i.e. animals display no spotting coat colour, indicated that presence of the Tobiano spotting gene, and its associated spotting coat colour pattern, is in the minority across the KBP breed. Genotyping for the Cremello Cream gene, which is semi-dominant i.e. Carriers (N/CR) display Cream but to a lesser extent to Homozygote Cream (CR/CR), identified Homozygote and Carrier percentages of 0.53% and 6.32% respectively. The majority of KBP samples (93.16%) genotyped as Non-Carriers for Cremello, hence just like Tobiano spotting - Cream coat colour is also expected to be in the minority across the KBP breed.

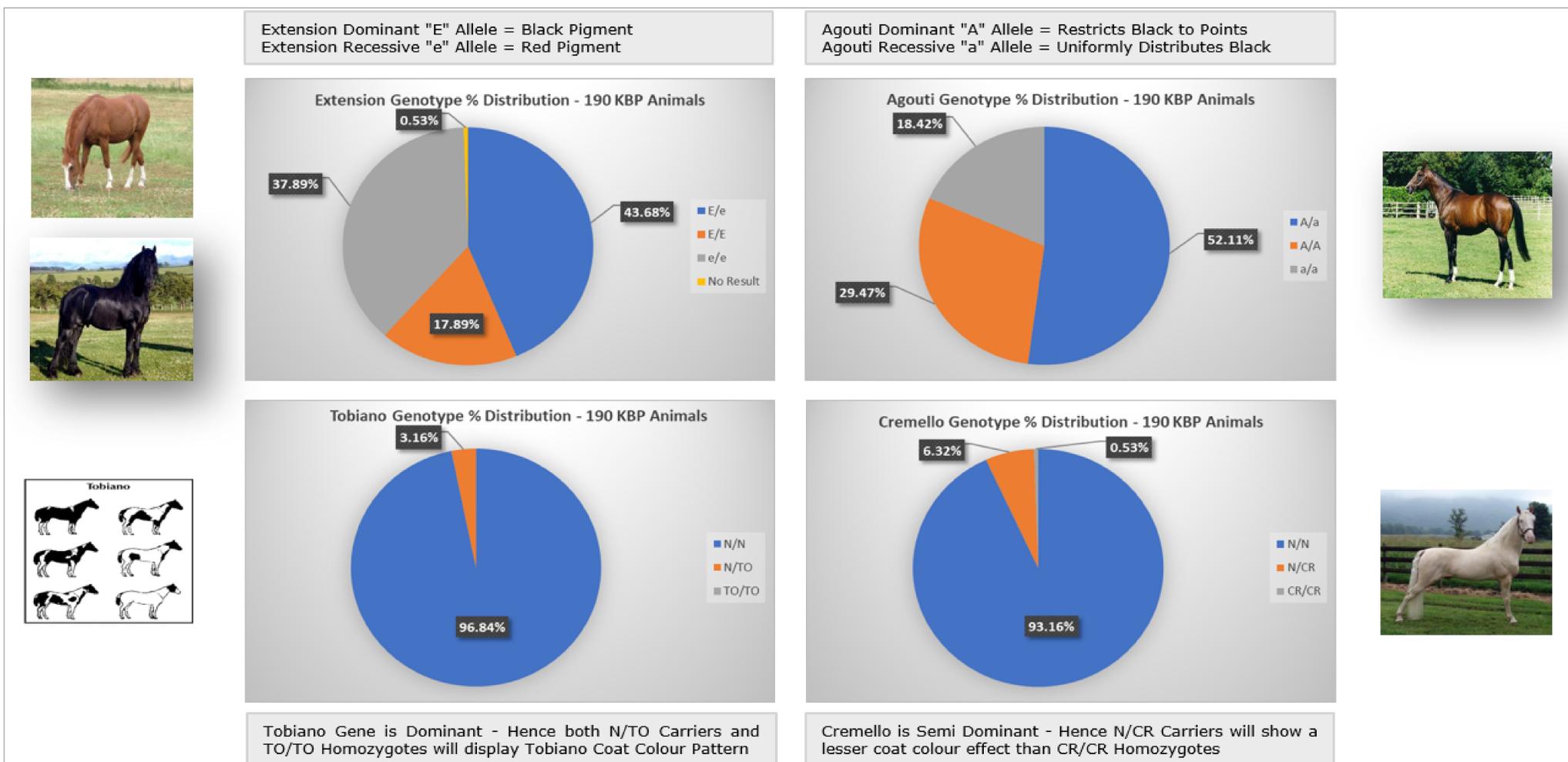


Figure 5: Percentage distribution of coat colour genotypes for 190 KBP animals, across Extension Black/Red, Agouti, Tobiano and Cremello coat colour genes.

## Project Review:

This project has seen the completion of a comprehensive genetic characterisation study for the Kerry Bog Pony (KBP) breed. This study utilised sample availability from a representative snapshot of 190 current active KBPs and performed individual genetic analysis to a level of molecular depth never previously undertaken for this breed. High quality SNP genotype profiles were established for 190 KBPs and subsequent analysis for key population genetic parameters, provided insights into the current genetic status of the KBP breed.

One such parameter was genomic inbreeding analysis, which demonstrated that the majority of KBP animals have a genomic inbreeding percentage of  $\leq 5\%$  and at comparative breed level, the KBP breed average of 3.4% proved to be at the lower end of the scale in comparison to other Equine breeds. Genomic relationship analysis indicated that the majority of KBPs were likely to be of First Cousin relations or less, and this observation supports low inbreeding findings. Small closed populations may suffer from increased inbreeding and with DAD-IS categorising the KBP breed as "At Risk", higher levels of average inbreeding may have been expected for the KBP breed, however comparative breed purity analysis offers an explanation here.

A hypothesised KBP breed signature was established for breed purity analysis and be it breed purity results may be subject to change depending on comparative breeds and analysis models used, a clear level of recent outcrossing was observed with Welsh Section A, Connemara, Shetland and New Forest Pony breeds. Such outcrossing may be an artefact of recent breeding attempts to maintain breed numbers or as a result of natural mating and can potentially expand a breed's gene pool, subsequently reducing inbreeding levels - which appears to be the case for the KBP breed. Consistent outcrossing introduces a risk of losing an original breed type, however a high average KBP breed purity (76%) was observed within males which offers a concentrated means of disseminating KBP type germ plasm across the breed as needed.

Coat colour genotypes associated with Chestnut, Bay/Brown or Black were observed across the KBP and the near even representation of all alleles for these base coat colour markers is in line with the wide coat colour spectrum observed across the KBP breed. Conflicting genotype and phenotype coat colour records for 15 animals requires further investigation i.e. phenotype vs genotype review, and these initial observations may provide merit to coat colour genotyping animals as part of KBP registration processes. A low percentage of both Tobiano and Cremello alleles were detected, indicating that animals of Spotting Pattern or Palomino/Cremello may be occasionally observed; but are not representative of the general coat colour genetics that currently exists across the KBP breed.

Individual animal reporting for the following metrics to the KBP society is underway 1) Genomic Inbreeding, 2) Genomic Relationships between Sire/Dam combinations (and predicted inbreeding for subsequent offspring) and 3) Coat Colour genotypes - and these metrics can be potentially used for current or future breed conservation management strategies for this rare native Irish breed.

## References:

- Petersen JL, Mickelson JR, Cothran EG, Andersson LS, Axelsson J, et al. (2013) Genetic Diversity in the Modern Horse Illustrated from Genome-Wide SNP Data. PLoS ONE 8(1): e54997. doi:10.1371/journal.pone.0054997
- Winton CL, Hegarty MJ, McMahon R, et al. Genetic diversity and phylogenetic analysis of native mountain ponies of Britain and Ireland reveals a novel rare population. Ecol Evol. 2013;3(4):934-947. doi:10.1002/ece3.507

## Funding:

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\* Average genomic inbreeding values for comparative breeds are based on a sample snapshot; hence indicative only of across breed averages and estimates do not account for possible SNP ascertainment bias, particularly within the Thoroughbred breed.