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## THAILAND PONY GENETICS PROJECT SUMMARY

### Investigators:

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### Purpose:

Genetic characterization of native Thailand Pony and application of molecular and management methods for preservation of a national genetic resource

### Experimental Design:

**Molecular Markers:** Genetic data are represented by DNA genotypes of 25 microsatellite markers (AHT4, AHT5, ASB2, ASB17, ASB23, HMS3, HMS6, HMS7, HTG4, HTG10, LEX33, TKY287, TKY294, TKY297, TKY301, TKY312, TKY325, TKY333, TKY337, TKY341, TKY343, TKY344, TKY374, TKY394, VHL20).

**Populations:** Hair samples with roots attached were collected *in loco* for representative 54 Mongolian Horses and 46 Thailand Ponies. Samples were transferred to the Veterinary Genetics Laboratory (VGL), University of California, Davis, CA, USA, for DNA genotyping and statistical analysis. Genotype data for 20 horse breeds (40 animals each), and the Przewalski horse (29 animals) were collected using samples from the VGL archive. The 20 populations represented a collection of ancient and modern European and North American breeds of both heavy and light horses, and ponies: Akhal-Teke, Andalusian, Arabian, Belgian, Clydesdale, Colombian Horse, Connemara, Friesian, Holsteiner, Lippizan, Morgan Horse, Norwegian Fjord, Paso Fino, Percheron, Peruvian Paso, Shire, Thoroughbred, Welsh Pony, Kiger Mustang, Rocky Mountain Horse.

**Statistical Analyses:** Standard metrics of genetic diversity for each breed that have been obtained are: allele frequencies, average number of alleles, average heterozygosity. Phylogenetic relationships among breeds were estimated by means of Nei's  $D_a$  genetic distance measure. To evaluate integrity of the sampled populations, assignment tests were performed with and without prior information of source breeds using the Bayesian method implemented in the computer program STRUCTURE. The computer program GENECLASS2 was also used for assignments tests based on the genotype likelihood method with simulation.

### Results and Discussion:

For purposes of this summary, results are focused on the Thailand Pony. The overall genetic diversity of the 23 horse populations is summarized in Table 1. Among the 23 populations, Mongolian Horse showed the overall highest diversity and mean number of alleles, followed by the Thailand Pony. These results indicate that Thailand Pony has not been subject to intensive selection by humans and thus retains high genetic diversity.

**Table 1. Summary of overall genetic diversity of 23 horse populations.**

Population	N	Expected Heterozygosity	Observed Heterozygosity	No. Alleles
Akhal-Teke	40	0.7288	0.7371	6.76
Andalusian	40	0.7276	0.7120	7.24
Arabian	40	0.6796	0.6600	5.68
Belgian	40	0.7238	0.6900	6.24
Clydesdale	40	0.6643	0.6460	5.56
Connemara	40	0.7543	0.7690	7.24
Friesian	40	0.5763	0.5780	4.60
Holsteiner	40	0.7613	0.7440	6.96
Lippizan	40	0.6967	0.6830	6.76
Morgan Horse	40	0.7546	0.7480	7.24
Norwegian Fjord	40	0.7100	0.6910	6.60
Paso Fino	40	0.7496	0.7530	7.96
Percheron	40	0.7200	0.7220	6.88
Peruvian Paso	40	0.7488	0.7570	7.44
Przewalski Horse	29	0.6356	0.5803	4.80
Shire	40	0.7108	0.7140	6.36
Thoroughbred	40	0.7269	0.7090	5.64
Welsh Pony	40	0.8004	0.7870	7.84
Kiger Mustang	40	0.7341	0.7493	6.96
Rocky Mountain	40	0.7253	0.7480	7.40
Colombian Horse	40	0.7451	0.7379	7.60
Mongolian Horse	54	0.8044	0.8032	9.72
<b>Thailand Pony</b>	<b>46</b>	<b>0.7939</b>	<b>0.8078</b>	<b>8.92</b>

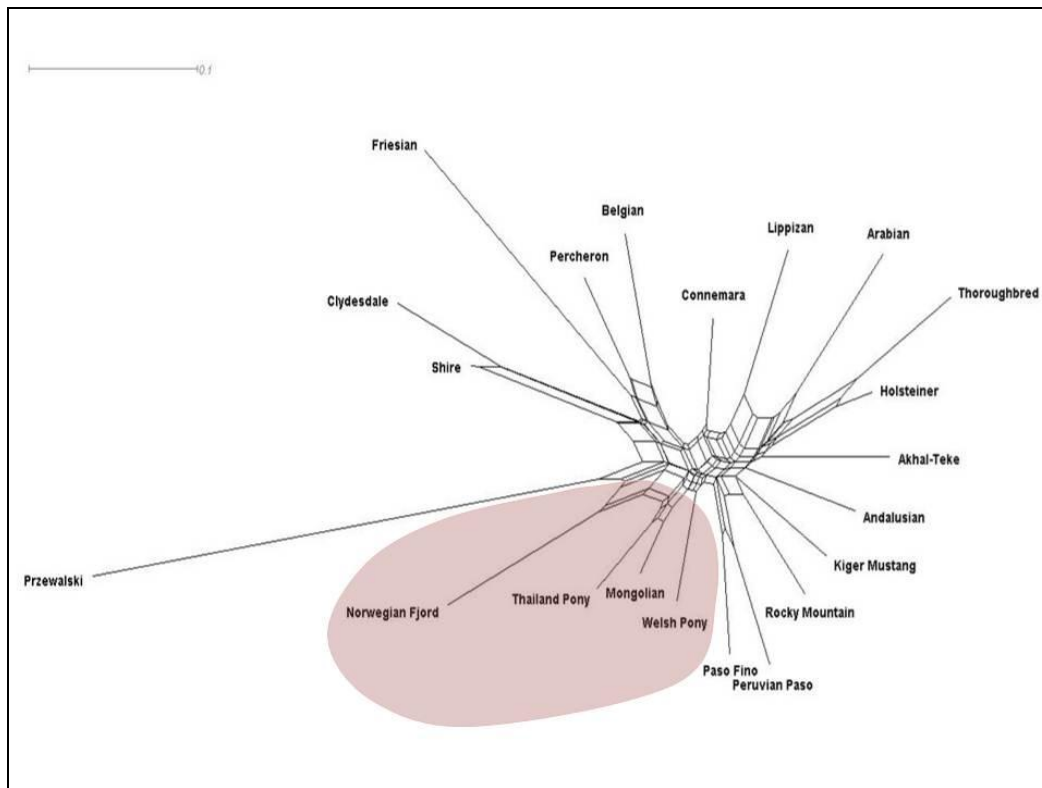
Examination of the alleles present in each of the 25 loci showed presence of a few rare variants found exclusively in Mongolian and Thailand Pony and in one instance, only in Thailand Pony (data not shown). This observation points to Thailand Pony as a reservoir of variants in these markers that may only be found among Asian horses. Because the markers used in the analyses have no functional biological role, no particular effort should be placed to preserve these rare variants, at the cost of other more relevant traits.

Genetic distance analyses showed that Mongolian Horse is the closest related breed of the Thailand Pony. The genetic distance metric  $D_a$  between Thailand Pony and Mongolian Horse is 0.08, whereas pairwise  $D_a$  values between Thailand Pony and the other breeds studied ranged from 0.12 (Welsh Pony) to 0.31 (Friesian). The phylogenetic tree depicted in Figure 1 shows the close relationship of Thailand Pony to the Mongolian Horse, and also a closer association with pony breeds such as Norwegian Fjord and Welsh Pony than any of the other breeds included in this study. Overall, this

analysis supports Thailand Pony of having ancestry among Asian horses, more likely from Mongolian Horse. In order to validate this conclusion, other Asian horse breeds would need to be investigated.

To test the integrity (genetic subdivision) of the 23 populations, a clustering analysis was performed with the software STRUCTURE without identification of source populations and with number of putative populations ranging from 2-25. The results of these analyses showed that the 23 breeds formed 18 clusters, 5 of which contained pairs of genetically related breeds: Paso Fino/Colombian Horse, Belgian/Percheron, Thoroughbred/Holsteiner, Clydesdale/Shire and Mongolian Horse/Thailand Pony. These results are in agreement with our finding above of close genetic relationship among Mongolian Horse and Thailand Pony. When source populations were defined as each of the 23 breeds, the Mongolian Horses had high values of genotype membership within their group (average 0.959), as did the Thailand Horses (average 0.978). Results of analyses using the likelihood with simulation method of GENECLASS2, were in agreement with those of STRUCTURE. Although source populations are given, results of assignment for Mongolian Horse showed that 65% of the horses were assigned only to the source population and 35% also had smaller but significant inclusion probability in the Thailand Pony, but no other population. For Thailand Pony, 22% of the horses were assigned only to the source population and 78% also had smaller but significant inclusion probability in Mongolian Horse. Given that there is currently no gene flow of Mongolian Horses into Thailand, these findings again reinforce the close genetic relationship among Mongolian Horse and Thailand Pony. They also provide evidence that the Thailand Ponies selected for this study represent a native population that is distinct but yet reflects its Asian origin and that has not been eroded through crossbreeding.

**Figure 1. Phylogenetic tree based on Da genetic distance among 20 breeds.**



One of purposes of the population structure analyses was to develop a database of reference breeds and genotypes for genetic evaluation of candidate animals for purposes of conservation of Thailand Ponies. As these native animals are not currently managed as a breed, i.e., there is no registry to record and validate pedigrees and no monitoring of breeding activities, it is important to use all tools available for the identification of the best representatives of the breed for inclusion in the conservation program being organized by the Lampang Pony Welfare Foundation. To this end, 3 candidate stallions and 2 candidate mares were submitted for genetic testing and population assignment. Population assignment results (Table 2) identified the 3 stallions and the Lampang mare #01 as good representatives of Thailand Pony and excluded one of the mares as a candidate given the low values of genotype probability obtained by all 3 methods used.

**Table 2. Assignment of LP01, LP02, LP03, Lampang Mare #01, Pimai Mare #01.**

Animal	Structure		Genotype Probability	Genotype Probability with Simulation	
	Membership Proportion			1 <sup>st</sup> Ranked Pop	TH
	TH	MO			TH
LP01 stallion	0.791	0.019	TH: 0.999	0.212	0.055
LP02 stallion	0.152	0.574	MO: 0.989	0.208	0.694
LP03 stallion	0.540	0.095	TH: 0.989	0.188	0.180
Lampang mare #01	0.784	0.064	TH: 0.997	0.129	0.096
Pimai mare #01	0.424	0.116	TH: 0.449	0.029	0.096

In conclusion, the analyses carried out thus far have characterized a native horse population - the Thailand Pony – which had not been previously studied. Native populations of livestock are at risk of disappearing across the globe and have been the subject of efforts to preserve these genetic resources. The native Thailand Pony is a working equid that has deep links with the history and culture of Thailand. While work still continues, the genetic analyses performed thus far support the existence of a population of animals with the necessary physical and genetic characteristics to represent a breed closely related to the ancient Mongolian Horse. Their continued presence in Thailand attests to their history of adaptation to and survival in the tropical environment of the country without much human interference. Efforts to preserve this equine population are well justified. Molecular and statistical methods that are already in place are available to assist in the identification of animals targeted for conservation.