10.1071/ZO13062_AC

Australian Journal of Zoology 2013, 61(5), 357-365

SUPPLEMENTARY MATERIAL

Microsatellite analysis of genetic variability in Waler horses from Australia

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Supplementary Table 1. Breeds and sampling background

Breed	N	Breed sampling
Waler (WA)	70	Samples were provided by the Waler Horse Owners and Breeders Association Australia Inc. (WHOBAA). Samples were collected from seven breeding stations as follows: WA1 (29 horses), WA2 (12 horses), WA3 (10 horses), WA4 (one horse), WA5 (7 horses), WA6 (nine horses), WA7 (one horses) and one unknown horse.
Irish Draught (ID)	46	Samples contributed by breeders for parentage testing at the Animal Genetic lab at Texas A&M University.
Thoroughbred (TB)	70	Samples contributed by breeders for parentage testing at the Animal Genetic lab at Texas A&M University.
Quarter Horse (QH)	66	Samples contributed by breeders for parentage testing at the Animal Genetic lab at Texas A&M University.
Andalusian (AN)	33	Samples contributed by breeders for parentage testing at the Animal Genetic lab at Texas A&M University.
Turkoman (TU)	82	Samples came from the Turkoman Horse breeders in the USA. Samples came to the Animal Genetic lab at Texas A&M University for parentage testing.
Argentine Criollo (AC)	25	Samples contributed by breeders for parentage testing at the Animal Genetic lab at Texas A&M University.
Syrian Arabian (SY)	100	Samples were collected from Syria where Arabian horse breeders volunteered to give their horse samples.
Exmoor Pony (EX)	70	Samples contributed by breeders for parentage testing at the Animal Genetic lab at Texas A&M University.
Cleveland Bay (CB)	90	Samples contributed by breeders for parentage testing at the Animal Genetic lab at Texas A&M University.
Welsh Pony (WP)	31	Samples contributed by breeders for parentage testing at the Animal Genetic lab at Texas A&M University.
Timor Pony (TM)	33	Samples provided by owners for study of origins of Timor Pony and for this study
Belgian Draft (BE)	26	Samples contributed by breeders for parentage testing at the Animal Genetic lab at Texas A&M University.
Dales Pony (DL)	86	Samples contributed by breeders for parentage testing at the Animal Genetic lab at Texas A&M University.
Shire (SH)	32	Samples contributed by breeders for parentage testing at the Animal Genetic lab at Texas A&M University.
Highland Pony (HL)	28	Samples contributed by breeders for parentage testing at the Animal Genetic lab at Texas A&M University.

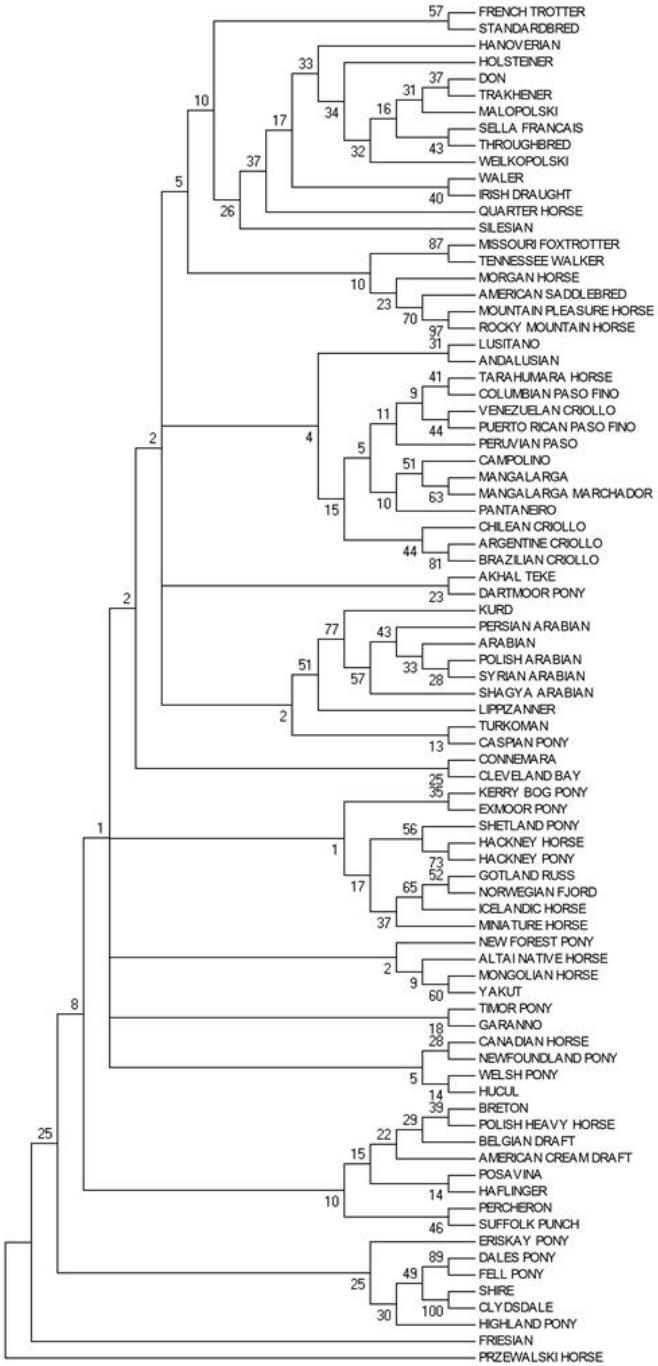
Supplementary Table 2. Information about microsatellite markers used in the study

ECA*	Marker	Primer Sequences 5'to 3'	Allele Size (bp)	Multiplex
4	LEX33	Forward: TTTAATCAAAGGATTCAGTTG	192-222	1
		Reverse: GGGACACTTTCTTTACTTTC		
24	AHT4	Forward: AACCGCCTGAGCAAGGAAGT	142-164	2
		Reverse: CCCAGAGAGTTTACCCT		
2	ASB17	Forward: ACCAGTCAGGATCTCCACCG	81-125	2
		Reverse: GAGGGCGGTACCTTTGTACC		
4	HMS6	Forward: GAAGCTGCCAGTATTCAACCATTG	155-169	2
		Reverse: CTCCATCTTGTGAAGTGTAACTCA		
9	HTG4	Forward: CTATCTCAGTCTTGATTGCAGGAC	126-142	2
		Reverse: CTCCCTCCCTCCCTCTGTTCTC		
30	VHL20	Forward: CAAGTCCTCTTACTTGAAGACTAG	85-109	2
		Reverse: AACTCAGGGAGAATCTTCCTCAG		
4	HTG7	Forward: CCTGAAGCAGAACATCCCTCCTTG	117-133	3
		Reverse: ATAAAGTGTCTGGGCAGAGCTGCT		
15	HTG6	Forward: CCTGCTTGGAGGCTGTGATAAGAT	79-105	3
		Reverse: GTTCACTGAATGTCAAATTCTGCT		
3	ASB23	Forward: ACATCCTGGTCAAATCACAGTCC	181-209	2
		Reverse: GAGGGCAGCAGGTTGGGAAGG		
15	ASB2	Forward: CACTAAGTGTCGTTTCAGAAGG	218-256	1
		Reverse: GCACAACTGAGTTCTCTGATAGG		
1	HMS7	Forward: CAGGAAACTCTCATGTTGATACCATC	171-189	2
		Reverse: TGTTGTTGAAACATACCTTGACTGT		
8	ATH5	Forward: ACGGACACATCCCTGCCTGC	126-148	2
		Reverse: GCAGGCTAAGGAGGCTCAGC		
9	HMS3	Forward: CCAACTCTTTGTCACATAACAAGA	152-180	1
		Reverse: CCATCCTCACTTTTTCACTTTGTT		
10	HMS2	Forward: ACGGTGGCAACTGCCAAGGAAG	216-244	3
		Reverse: CTTGCAGTCGAATGTGTATTAAATG		
21	HTG10	Forward: CAATTCCCGCCCCACCCCCGGCA	87-113	2
		Reverse: TTTTTATTCTGATCTGTCACATTT		

*Locus locations in the horse genome

Supplementary Table 3. Nei's genetic distances (D) between Waler and other 82 breeds Fifteen Breeds close to Waller are in bold.

Breed	D	Breed	D	Breed	D
Akhal Teke	0.2892	Gotland Russ	0.5967	Peruvian Paso	0.2742
Altai Native Horse	0.1469	Hackney Horse	0.3312	Polish Arabian	0.2906
American Cream Draft	0.298	Hackney Pony	0.3116	Polish Heavy Horse	0.3205
American Saddlebred	0.2318	Haflinger	0.3736	Posavina	0.3016
Andalusian	**0.2352**	Hanoverian	0.0974	Przewalski	1.0365
Arabian	0.2853	**Highland Pony**	**0.362**	Puerto Rican Paso	0.3129
Argentine Criollo	**0.232**	Holsteiner	0.1656	**Quarter Horse**	**0.0741**
Belgian Draft	**0.4179**	Hucul	0.3306	Rocky Mountain Horse	0.1784
Boerperd	0.2388	Icelandic Horse	0.4633	Sella Francais	0.1231
Brazilian Criollo	0.1574	**Irish Draught**	**0.0872**	Shagya Arabian	0.2733
Breton	0.4558	Kerrybog Pony	0.2402	Shetland Pony	0.2141
Campolino	0.2626	Kurd	0.2159	**Shire**	**0.3244**
Canadian Horse	0.2358	Lippizanner	0.2567	Silesian	0.206
Caspian Pony	0.2669	Lusitano	0.2335	Standardbred	0.2182
Chilean Criollo	0.2671	Malopolski	0.0968	Suffolk Punch	0.2607
Cleveland Bay	**0.354**	Mangalarga	0.2774	**Syrian Arabian**	**0.2283**
Clydsdale	0.4368	Mangalarga Marchador	0.2828	Tarahumara Horse	0.1531
Columbian Paso Fino	0.2299	Miniature Horse	0.2779	Tennessee Walker	0.1988
Connemara	0.2081	Missouri Foxtrotter	0.1388	**Thoroughbred**	**0.1297**
Dales Pony	**0.3257**	Mongolian Horse	0.249	**Timor Pony**	**0.3899**
Dartmoor Pony	0.23	Morgan Horse	0.1741	Trakhener	0.1776
Don	0.1826	Mountain Pleasur_Horse	0.1134	**Turkoman**	**0.1557**
Eriskay Pony	0.5307	Newforest Pony	0.159	Venezuelan Criollo	0.1324
Exmoor Pony	**0.4373**	Newfoundland Pony	0.2723	Weilkopolski	0.0729
Fell Pony	0.3273	Norwegian Fjord	0.3843	**Welsh Pony**	**0.2448**
French Trotter	0.4405	Pantaneiro	0.2504	Yakut	0.2239
Friesian	0.7894	Percheron	0.3267		
Garanno	0.1359	Persian Arabian	0.2804		



Supplementary Figure: Dendogram of the genetic relationship among 83 horse breeds.

Supplementary Text 1. Amplification conditions used in the study

We genotyped 2705 horses by using 15 microsatellite markers. The amplification of these microsatellites was done in multiplex PCR reactions and performed in a total volume 20µl reactions containing 30 ng of genomic DNA, 0.8 µM of primers, 1xPCR buffer, 2.5mM of MgCl2, 0.2 mM of dNTPs and l unit of AmpliTaq (PE Applied Biosystems, MA). A hot start procedure was used in which DNA template and primers were combined and heated at 95°C for 10 min. The temperature was then lowered and held at 85°C for 10 minutes for the addition of the remaining reagents. Thirty two cycles of 1 minute at 95°C, annealing 56°C for multiplex 1 and 60, for multiplex 2 and 3 for 30 seconds and 72°C for 45 seconds, then cycling was completed with a final extension at 72°C for 10 minutes. The PCR products were separated by electrophoresis on a 6% polyacrylamide gel using the ABI PRISM 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). Fragment sizes of microsatellite alleles were determined using the STRand computer software (www.vgl.ucdaviess.edu/STRand). Alphanumerical nomenclature was used for allele size designation in accordance with the International Society for Animal Genetics.