

Table 1 PCR primers for the amplification of canine *TPP1* exons.

Forward primer	Sequence (5'–3')	Reverse primer	Sequence (5'–3')	T _M (°C)	Product size (bp)
TPP1_Ex1,2_F	CGGTGGAACATAGGGTTCAT	TPP1_Ex1,2_R	GAGCTGGTAATGGGGTGAAG	60	347
TPP1_Ex3_F	ATCCTACCCAGGTCTCAGC	TPP1_Ex3_R	TATGTGCACCATCCCACACT	60	328
TPP1_Ex4,5_F	CTCCTGGTTGAGGTCCAAAA	TPP1_Ex4,5_R	CCCCTGACTCTGTCTGCTTC	60	596
TPP1_Ex6_F	GGGTGGGATCAATGGTAAAGT	TPP1_Ex6_R	CAGGGGATGTTGTTCCAGTT	60	365
TPP1_Ex7_F	CCTGGGTTCTCACCTCTAA	TPP1_Ex7,8_R	TGATCACCACCTCACCACCAC	60	350
TPP1_Ex8,9_F	TGACAACGTCCTTGTGTTCC	TPP1_Ex8,9_R	GTCAAGGCAAAGGTTCCAGA	60	604
TPP1_Ex10,11_F	ATTTTGGAGGTCCCCTTGAG	TPP1_Ex10,11_R	CTTAGAGGAGGCAGGGGTTTC	60	507
TPP1_Ex12,13_F	CCCCAAATCCAATCACTTTG	TPP1_Ex12,13_R	AGAGACCAAGCCACCAGCTA	60	553

Table 2 Polymorphisms in the canine *TPP1* gene.

Position within <i>TPP1</i>	Nucleotide position ¹	English Setter (reference sequence) ¹	Boxer (Tasha) ²	Tibetan Terrier (non-affected) (n = 1)	Tibetan Terrier (NCL-affected) (n = 2)	Polish Owczarek Nizinny (NCL-affected) (n = 1)
Intron 2	284–287	CTGC	::::	::::	::::	::::
Intron 2	303	G	T	T	T	T
Intron 3	703	A	G	G	G	G
Intron 4	2084	A	G	A	A	G
Intron 4	2110	T	G	G	G	G
Intron 4	2145	C	T	C	C	C
Exon 7	3064	T	C	T	T	T
Intron 8	3566	C	A	A	A	A
Exon 11	4334	T	C	T	T	C
Intron 11	4826	T	C	T	T	T
Intron 11	4829	C	T	C	C	C
Exon 13, non coding region	5300	C	G	G	G	G
Exon 13, non coding region	5302	C	G	G	G	G

¹Positions are numbered according to GenBank accession no. AF114167.

²Publicly available dog genome sequence from a boxer named Tasha.

were breed specific and because of the fact that both NCL-affected and non-affected animals showed identical genotypes, these polymorphisms appear to be unrelated to the NCL phenotype. Our data thus indicate that these polymorphisms in the canine *TPP1* gene can be excluded as candidate mutations for the NCL phenotype in the Tibetan Terrier and PON dogs.

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doi:10.1111/j.1365-2052.2005.01245.x

Phylogenetics of the European Dahomey miniature cattle based on mitochondrial D-loop region DNA sequence

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Accepted for publication 24 January 2005

Source/description: The origin and taxonomic status of domesticated cattle in Africa is still controversial. Zebu and taurine breeds are differentiated primarily on the presence or absence of a hump and have been recognized as separate species, i.e. *Bos taurus* and *Bos indicus*. In this context the direct ancestor of the European Dahomey miniature cattle is still unclear. At the beginning of the 20th century, the Dahomey miniature cattle

was imported from Africa into Europe and today, there are approximately 50 breeders in Germany, The Netherlands, Switzerland, Austria, France and Czech Republic. However, there is currently no information regarding the origin of the European Dahomey population. We have examined the mitochondrial D-loop region, because it is known to be the most variable mtDNA region and therefore useful in phylogenetic studies.¹ The data obtained were compared with published DNA sequences of European and African cattle breeds to assess the relationship of the European Dahomeys.

Primer sequences: Primer 1 D-loop u: 5'-AAATGTAACACGACGCGCCAGTAATCCAATAACTCAACAC-3'

Primer 2 D-loop l: 5'-AAACAGGAAACAGCTATGACCATCATCTAGGCATTTTC-3'

The underlined part of the primer contains the binding site for a sequencing primer whereas the 3'-part matches to the mtDNA.

PCR conditions and sequencing: Amplification was performed in a final volume of 20 µl in 10xPCR buffer (15 mM MgCl₂, pH 8.3) and Q-solution, 100 µM of each dNTP, with 1 U *Taq* Polymerase (Qiagen GmbH, Hilden, Germany) and 10 pmol of each primer. Four microlitres of genomic DNA (5 ng/µl) was added to the PCR mix. The amplification was carried out with initial denaturation at 95 °C for 10 min, followed by 35 cycles of 94 °C for 40 s, 52 °C for 40 s and 72 °C for 45 s in a Hybaid thermocycler (MWG Biotech, Ebersberg, Germany). PCR products were purified using the QIAEX II Gel Extraction Kit (Qiagen GmbH) according to the manufacturers' instructions. Sequencing was performed using the ABI-Prism™ Dye Kit V3 (Applied

Biosystems, Weiterstadt, Germany) in a 10-µl volume containing 2 µl purified PCR product (25 ng/µl) and 5 pmol of the reverse and forward sequencing primer (underlined part of primers 1 and 2). Sequencing reactions underwent 27 cycles of 30 s at 94 °C, 30 s at 50 °C and 3 min at 60 °C using a Techne thermocycler (Techne, Burkhardtendorf, Germany). The dye terminators were removed by Sephadex-G45 column purification (Millipore, Schwalbach, Germany). Sequencing reactions were electrophoresed for 2 h on an ABI Prism® 3100 genetic analyzer (Applied Biosystems) according to the manufacturers' instructions. Phylogenetic and molecular evolutionary analyses were conducted with the resulting sequence using MEGA version 2.1.² The sequence has been deposited in GenBank under accession number AY327891.

Polymorphisms: We have analysed a 323-bp fragment of the mtDNA of 15 Dahomey miniature cattle and detected six polymorphisms (Table 1). The 323-bp amplicons corresponding to positions 15 961–16 284 of the mitochondrial D-loop region were compared with published D-loop regions of Limousin, Ayrshire, Angus, Charolais, Hereford, Simmental, Jersey, Butana, Kenana and N'Dama.^{3,4} The positions refer to the consensus bovine mtDNA sequence (accession no. MIBTXX).¹ The first SNP, a transition A → G (position 16 022) present in all investigated European cattle breeds, e.g. Simmental and Limousin, was also observed in two African breeds, i.e. Kenana and N'Dama. Dahomey and Butana differed at this nucleotide position. Furthermore, three transitions (T → C at position 16 050, C → T at position 16 112 and C → T at position 16 255) were shared between the European breeds. One base substitution, a C → T transition at position 16 247 was characteristic for Butana and Jersey cattle. In N'Damas a single A → G transition at position 16 057 was found.

To construct phylogenetic trees and calculate sequence divergence, we used the computer program as described.² Levels of sequence divergence among European and African breeds ranged from 0 to 0.0156 (mean = 0.00723). The highest distance (0.0156) was between Dahomey and Jersey. As seen in the phylogenetic tree (Fig. 1), the Dahomey is separated from other European lineages. Previously it has been shown that most of the African diversity is clustered around a

Table 1 Alignment of variable mitochondrial D-loop region positions.

	Variable nucleotide positions ^a					
	1	1	1	1	1	1
	1	1	1	1	1	1
	6	6	6	6	6	6
	0	0	0	1	2	2
	2	5	5	1	4	5
	2	0	7	2	7	5
MIBTXX ¹	G	C	G	T	C	T
Dahomey	A	T	.	C	C	C
Kenana ³	.	T	.	C	C	C
N'Dama ³	.	T	A	C	C	C
Butana ³	.	.	.	C	T	C
Simmental ³
Hereford ³
Charolais ³
Angus ³
Limousin ³
Ayrshire ⁴
Jersey ⁴	T	.

^aNucleotide positions refer to EMBL database entry accession no.

MIBTXX.¹

Accession no. L27729 (Kenana), L27731 (N'Dama), L27714 (Butana), L27735 (Simmental), L27725 (Hereford), L27717 (Charolais), L27712 (Angus), AF034446 (Limousin), AF034440 (Ayrshire), and AF034439 (Jersey).

^{1,3,4}See references.

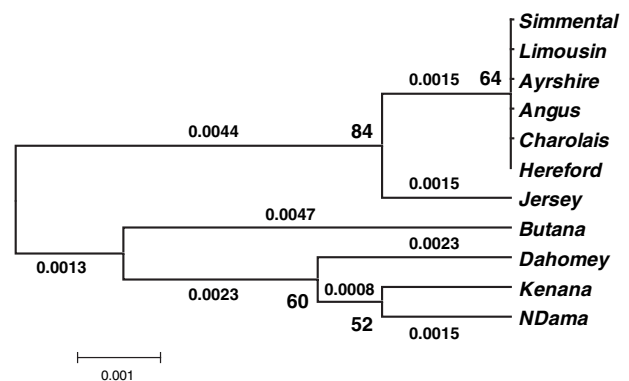


Figure 1 Phylogenetic relationships of 11 cattle breeds. UPGMA-tree obtained from mtDNA sequence data. (Kimura 2-parameters: transitions and transversions included). Bootstrap values and distances are indicated.

haplotype that is absent from European breeds.⁵ The African Dahomey lineage is more isolated from Butana than from other tested breeds. Separation from these two main African lineages indicates that the Dahomey is a special lineage. Due to the nucleotide variations in the D-loop region, we conclude that the European Dahomey cattle do not have a single ancestor. As the sample size was small, we cannot exclude additional base substitutions at other positions. However, based on the results shown here European Dahomeys seem to coexist between the investigated Butana on the one hand, and the N'Dama and Kenana on the other. The absence of substitutions similar to those identified in *Bos taurus* might be an indication for the fact that Dahomeys bred in Europe are more likely originating from African cattle breeds, e.g. Samba, rather than from crosses with other European cattle breeds.

Acknowledgements: The study was supported by the Erxleben Research & Innovation Council (ERIC-BR1959-2001-08).

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doi:10.1111/j.1365-2052.2005.01256.x

Linkage and radiation hybrid mapping of the porcine *MPZ* gene to chromosome 4q

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Accepted for publication 31 January 2005

Source and description: Myelin protein-zero is the major structural protein of peripheral myelin. Expression of the *MPZ* gene is restricted to Schwann cells.¹ In humans, defects in function of the *MPZ* gene product are associated with the Charcot-Marie-Tooth disease type 1, Dejerine–Sottas syndrome and congenital hypomyelinating neuropathy, as well as several types of CMT2. One of the symptoms is characterized by slowly progressive distal muscle atrophy and weakness.² The human *MPZ* gene consists of six exons³ and was physically mapped 130 kb

centromeric to the Fc immunoglobulin gene cluster in band 1q22.⁴ Polymerase chain reaction (PCR) primers (F1 and R1) were designed based on the human genomic *MPZ* sequence (<http://www.ensembl.org>; accession no. ENSG00000158887; exons 2 and 6). Primers F2 and R2 were designed based on the sequenced porcine fragment.

Primer sequences: Forward F1: 5'-ATC TCC TTC ACC TGG CGC TAC C-3'

Reverse R1: 5'-GGC CCG CTA ACC GCT ATT TC-3'

Forward F2: 5'-AAA GAG AAC TTG GGC TAA GA-3'

Reverse R2: 5'-GTG AAA GTG CCG TTG TC-3'

PCR conditions and sequencing: PCR with primers F1 and R1 was performed in 25- μ l reaction volume using 100 ng of porcine genomic DNA, standard reaction buffer, 1.25 mM MgCl₂, 200 μ M each dNTP, 10 pmol of each primer and 1 U *Taq* polymerase (Top-Bio, Prague, Czech Republic). Cycling conditions were as follows: 95 °C for 1 min, 30 cycles of 94 °C for 1 min, 55 °C for 30 s, 72 °C for 90 s, and a final extension of 10 min at 72 °C. The PCR fragment F1–R1 (1307 bp from Meishan and Piétrain pigs) was sequenced using a Thermo Sequenase Cy5 Dye Terminator Sequencing Kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and an ALFexpress Sequencing System (Pharmacia Biotech, Uppsala, Sweden). The sequences (without primer sequences) have been deposited in the EMBL database under accession numbers AJ875404 and AJ876546 for Meishan and Piétrain, respectively. The coding part of the obtained sequence (partial exons 2 and 6, and complete exons 3, 4 and 5) shows 92% sequence identity with the orthologous human sequence (<http://www.ensembl.org>; accession no. ENSG00000158887). PCR amplification of the F2–R2 fragment (325 bp of intron 2 and exon 3) was performed under the following conditions: 10 μ l reaction, 50-ng genomic DNA, reaction buffer, 1 mM MgCl₂, 200 μ M each dNTP, 4 pmol of each primer and 0.5 U *Taq* polymerase. Cycling conditions were: 95 °C for 1 min, 33 cycles of 94 °C for 1 min, 54 °C for 30 s and 72 °C for 45 s, and a final extension of 5 min at 72 °C.

Polymorphism, Mendelian inheritance and allele frequencies: The F1–R1 fragments from four pigs (Meishan and Piétrain) were sequenced and seven SNP were identified in positions 60 (T–C), 182 (G–A), 210 (T–C), 455 (T–C), 497 (C–T), 1081 (G–C) and 1114 (G–A) of EMBL AJ875404. The SNP 455 (T–C) is in coding sequence, but the mutation is silent. One SNP (position 210) is in the recognition sequence for enzyme *Rsa*I. For polymorphism testing the F2–R2 primers were used. The

Table 1 Allele frequencies of SNP 210 in the porcine *MPZ* gene.

Breed	No. of animals	Allele	
		C	T
Large White	14	0.25	0.75
Landrace	12	0.67	0.33
Piétrain	22	0.09	0.91
Black Pied Prestice	7	0.36	0.64
Czech Meat Pig	14	0.39	0.61
Hampshire	6	0.25	0.75
Meishan	12	0.88	0.12