BRIEF NOTES

Eight new dinucleotide microsatellite loci in turkey (*Meleagris gallopavo*)

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Source/Description: A phage library was constructed in dephosphorylated *Bam*HI digested M13 from size-selected (220–1000 bp) turkey genomic DNA restricted with *Mbo*I. The library was screened with ^{32}P-labeled $(CA)_{16}$ and $(GT)_{16}$ probes. Positive clones were sequenced with an automated DNA sequencer (ABI 373) at the **A**dvanced **G**enetic **A**nalysis **C**enter, College of Veterinary Medicine, University of Minnesota. All sequences were queried against GenBank by BLAST searching. PCR primers were designed interactively with Primer v.3 (Whitehead Institute for Biomedical Research, http://www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi).

PCR Conditions: Oligonucleotide primer-pairs for each locus (Table 1) were optimized for PCR amplification by testing over a range of annealing temperatures (50–60 °C). PCR reactions (12 μl total volume) included 20–25 ng genomic DNA, 1·5 mM $MgCl_2$, 2·5 pmol each primer, 100 μM dNTP, and 0·35 U Hotstar *Taq* polymerase (Qiagen, CA). Amplifications were performed in a MJ Research thermal cycler under the following reaction conditions: 15 min at 94 °C; 30 cycles of 30 s at 94 °C, 30 s at annealing temperature, 30 s at 72 °C; and a final extension of 5 min at 72 °C.

Polymorphism and allele size: Polymorphism at each locus was determined by examining 12 individuals from two distinct populations. Our sample included six birds (four males and two females) housed at the University of Minnesota (Nicholas commercial line) and six birds (three males from two male production lines and three females from a single female production line) provided by Nicholas Turkey Breeding Farms, CA. DNA fragments for each locus were amplified and labeled for electrophoresis by substituting ^{32}P-dATP (0·3 pmol) in the PCR reaction. PCR products were denatured at 94 °C and electrophoresed through 7% acrylamide gels. After autoradiography, allele sizes were determined by comparing amplified fragments to internal size markers (M13 sequencing reaction). Product sizes and number of alleles at each locus are given in Table 1. Six of the eight loci were polymorphic in the individuals examined. The number of alleles ranged from 1 (*MNT-5* and *MNT-7*) to 6 (*MNT-1*) with an average of 2·8 alleles per locus. Five of the eight primer pairs (*MNT-1,3,5,6,* and *7*) produced a single appropriate-sized DNA fragment in PCR reactions with chicken genomic DNA (*Gallus gallus*) indicating their potential usefulness as markers in this species in addition to the turkey.

Chromosomal locations: Unknown

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Table 1. Turkey microsatellite primer information. The Genbank accession number, type and length of repeat, primer sequences, TM (PCR annealing temperature) of the primers, observed product size and number of alleles are included for each locus

Locus	Accession number	Repeat length	Forward primer	Reverse primer	TM	Product size (bp)	No. of alleles
MNT-1	AF176506	$(CA)_{26}$	ATCTCCCTCAGGCAGGTATC	AGACTCTTGTGGCCTGGAG	56	121–154	6
MNT-2	AF176507	$(CA)_{22}$	CTACGCCCCTTAGTATTGTGAC	TGCCTAGCTGTAAGCAAGTCTC	56	105–166	4
MNT-3	AF176508	$(CAGA)_8$ + $(CA)_{18}$	GATCACAGTCACACTCAAATGC	CCCAGTTTGGAGAGACATTC	56	125–140	3
MNT-4	AF176509	$(CA)_{21}$	CGACACTCGAAAGGTGTTC	AGCAGCTTTCATCCCATTTG	58	146–150	2
MNT-5	AF176510	$(CA)_{12}$	TGCTATGATATAAAACACCTCTGG	AAAACTTCTTGTGGCTTTCTCC	56	181	1
MNT-6	AF176511	$(CA)_{44}$ imperfect	CAGAGAGATTTACCGTCCTTTG	ACCCACAGGATGAAGGAATG	56	154–229	4
MNT-7	AF176512	$(TG)_{11}$ + $(TG)_7$ + $(TG/TA)_{12}$	TGGTAAATTGCGATTCTTGC	TAAGAGAAAGAGGGCCAATG	54	199	1
MNT-8	AF176513	$(CA)_{18}$	GGTGAATTGGCAGACATCAG	AGTTATTGCCAGTGCCTGAG	56	158–160	2

Genetic variations in the ovine calpain regulatory subunit

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Source/description: The ovine calpain regulatory subunit for both the micro and millimolar calcium dependent protease large subunit was screened with primers based on the published bovine cDNA sequence[1] (GenBank accession no. J05065). Primer selection took into account probable exon/intron boundaries, which were assigned after aligning the bovine cDNA sequence with human cDNA sequences for which exon/intron boundaries had been determined[2]. The forward primer corresponded to positions 262–280 and the reverse primer to positions 324–345 of bovine sequence J05065. The primer sequence locations coincided with positions in exons 3 and 4 of the human sequence[2].

PCR primer sequences:

5′ primer: GCG GCT GCC CAG TAC AAC C

3′ primer: CCG GAC CTC CTC ACT CTC ATT G.

PCR conditions: A total volume of 30 μl PCR mixture using 1× reaction buffer (10 mM Tris, pH 8·3, 50 mM KCl, 0·1% Triton X-100, 1·5 mM $MgCl_2$), 10 μM of deoxynucleoside triphosphates, 10 pmol of each primer, 50 ng genomic DNA, and 2 units of *Taq* DNA polymerase (BRL, Grand Island, NY) was used. Samples were submitted to 35 cycles of amplification in an MJ Research PTC-200 thermal cycler (MJ Research Inc, Watertown, MA). Each cycle consisted of 94 °C for 1 min for denaturation, 61 °C for 1 min for annealing, and 72 °C for 1 min for polymerization.

Analysis of PCR-generated fragments: The amplified fragment of the ovine calpain regulatory gene was visualized on a 1·2% agarose gel. The size of the fragment was estimated to be ≈ 400 bp. For SSCP (single strand comformation polymorphism), 8 μl of the PCR products were diluted with 8 μl of distilled water and 16 μl of loading buffer (0·25% bromophenol blue, 0·25% xylene cyanol FF, 30% 33 Glycerol). The mixtures were heated at 95 °C for 5 min, and placed on ice immediately. A total of 32 μl of mixture was adapted for SSCP with a 0·5 × MDE (mutation detection enhancement, FMC, Rockland, ME) polyacrylamide gel. Electrophoresis was conducted at 20 °C for 14 h in 0·5 × TBE buffer at 200 V after which bands were visualized by staining with ethidium bromide for 10 min

Polymorphism: Fifty purebred Polypay sheep from the Ohio Agricultural Research and Development Center were screened. Two alleles (*A* and *B*) and three resultant genotypes were observed using SSCP (Fig. 1). Frequencies for allele *A* and *B* were calculated as 0·11 and 0·89, respectively.

Mendelian inheritance: Codominant Mendelian segregation for the two alleles was observed in two half-sib Polypay families.

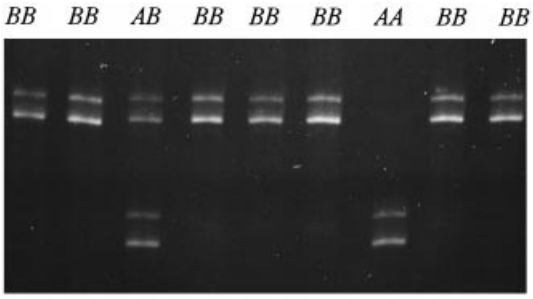

Fig. 1. PCR-SSCP polymorphism in the ovine calpain regulatory gene demonstrating the three genotypes (*AA, AB* and *BB*).

Chromosomal location: The location of the ovine calpain regulatory gene is not known. However, the human calpain regulatory gene, CAPN4, has been mapped to chromosome 19[3].

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Twenty-one new equine dinucleotide repeat microsatellites

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Source/Description: Horse genomic DNA was digested with restriction enzyme *Mbo*I and size selected by gel electrophoresis for fragments from 200 to 1200 bp. The digested size-selected DNA was then ligated into the *Bam*HI site of the M13 phage vector. Clones containing a microsatellite were identified by screening the library with [^{32}P] 5′ end labeled oligo $[dCA]_{16}$ and oligo $[dGT]_{16}$ probes. DNA was isolated from positive plaques and the inserts were sequenced on an ABI-377 sequencer at the Advanced Genetic Analysis Center, College of Veterinary Medicine, University of Minnesota. Primer pairs were developed from the sequences using the PRIMER program (Version 0·5; M.J. Daly, S.E. Lincoln and E.S. Lander, unpublished data).

PCR Conditions: PCR reactions contained 25·0 ng DNA, PCR Buffer (Boehringer), 1·5 mM $MgCl_2$, 5 pmol each primer, 25 μM each of dCTP, dGTP, and dTTP, 6·25 μM dATP, 0·125 μCi [α-^{32}P] dATP and 0·45 U *Taq* polymerase (Boehringer) in a final volume of 15 μl. The PCR reactions were performed in 96 well plates for 30 cycles with initial denaturation at 92 °C for 2 min; 30 cycles of 92 °C for 30 s, 54–62 °C for 30 s, and 72 °C for 30 s; and a final extension at 72 °C for 5 min using a MJ Research thermocycler (See Table 1). These reaction products were electrophoresed through 7% acrylamide denaturing gels on BioRad SequiGen GT 38 × 50-cm plate sequencing gel units and allele sizes detected through autoradiography.

Polymorphism and allele size: DNA from 12 stallions of the Equine Genome Mapping Workshop International Reference Family, and three horses (one stallion, two mares) from the parental generation of the Newmarket Reference Family, was amplified and polymorphic markers were identified (Table 1). Allele sizes were determined by comparison to the M13 sequence ladder. The number of alleles of a given marker ranged from 2 to 7, and the number of horses that were heterozygous ranged from 1 to 12.

Chromosomal locations: Unknown

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Polymorphic microsatellites associated with the equine *CKM* and *CMA1* genes

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Table 1. Equine microsatellite marker information

Marker	GenBank	Forward primer	Reverse primer	PCR temp.	No. alleles	Size range	Heterozygosity
UMNe50	AF91688	GTTGGAAGAGAACAACGAAAGG	GCTAAATTTCACTCCATCAGGG	60	3	121–131	0·55
UMNe51	AF91689	TCTCTGCTCCAGATGGCC	AACTTTCTCATCCCCAACCC	62	2	158–160	0·08
UMNe52	AF91690	TGTCTGCTGACAGATGAATGG	CATGGCTGAATAATATTCCGTG	60	3	88–104	0·4
UMNe54	AF91691	AACTCACTTCTTCCTGCTCG	CCAAGGCTCTTTGCCAAGAC	56	4	107–127	0·2
UMNe56	AF91692	TCTGTCTGCAGCTAAAGAGGC	GCGGGGTACATAAGACTGTAGC	60	3	193–201	0·2
UMNe58	AF91693	GATCCAAGACTTGAAGGTTAGC	TTTCCTCACCATCCTCCTTGAC	60	7	143–165	0·2
UMNe60	AF91694	TGTGGCAGGAAAAACACATG	CCATAATCCATGAGCCTATTCC	60	4	146–154	0·08
UMNe61	AF91695	CCGCCATCATTACTAAGCAG	TCTTTTCCTCCAATCCTACTCC	60	7	123–145	0·8
UMNe63	AF91696	GGATTTTCTTCTTTTGAATGGC	TTTACAATAGCCAAGATGCGG	60	3	128–142	0·46
UMNe64	AF91697	TTGCTTTTCTTCCTACACTCCC	TTGACCTAAAGCTCAATGTGTG	60	2	145–147	0·17
UMNe65	AF91698	TCCTTCCACTCCCCTCAAC	TCCCTGAAAAACCTTGGTTG	60	6	126–146	0·8
UMNe66	AF91699	GAATCCCATCTTTCCTTTCAG	ACGTGGAGAATTATCCTGCG	60	3	122–126	0·33
UMNe67	AF91700	TCCTTACCCCTTTGGAGATG	CAATGGTGTGCTCCATGAAG	54	3	171–175	0·5
UMNe68	AF91701	AATTTCAAACTCCAGCTCTTGC	GGGCCCAAGAATAAAGAAGG	58	3	121–133	0·47
UMNe69	AF91702	CCTCTTAAGGATGCTCACAGTG	TGCATGGGTGTATGGGTATG	60	3	122–130	0·43
UMNe70	AF91703	TGGGCATTATTTCACAGTATGC	TACATTAGGCCTGGAATGGG	58	2	150–152	0·5
UMNe71	AF91704	GGAAATTTGGGAGACAGTATGC	GGCAGAATTGTGACTAGAACCC	62	6	120–154	0·67
UMNe74	AF91705	CGATGGATGTGCTGTAAACG	TGCTGCCTTCTCCCTCAC	62	2	139–143	0·47
UMNe76	AF91706	CCCTCAGGTTGAGGACTCAG	AGGTGACAACCTGGATTTGC	60	3	98–102	0·54
UMNe77	AF91707	CAATGGGGACTTCTCAGATAGC	AGGGAGGTTGAAGAGTTTACCC	60	5	135–143	0·54
UMNe78	AF91708	CCTATTGCAAATGATTCACACC	AGAGATTTCAGGGAAACATTCC	60	5	134–152	0·42

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Source/description: DNA fragments amplified from a Thoroughbred horse with gene-specific universal mammalian sequence-tagged site primers[1] for *CMA1* (Chymase-mast cell) and *CKM* (Creatine Kinase-Muscle) were cloned and sequenced[2]. BLAST searches of GenBank at the National Center for Biotechnology Information internet server (http://www.ncbi.nlm.nih.gov/) with the sequences obtained from equine *CMA1* and *CKM* revealed homologies of 89% and 90% with the respective homologous human loci. STR sequences were identified within the fragments amplified from these equine type I genes. The repeat sequences found in the *CKM* and *CMA1* fragments were $(CA)_{11}(N)_{13}(CA)_4$ and $(TG)_2(CT)_3GT(CT)_4GT(CT)_8$, respectively. PCR primers (Table 1) were designed to amplify these repeat sequences and reaction conditions were optimized in MJ Research thermocyclers (PTC-100).

PCR conditions: Amplifications were carried out using 2 μl of serum or hair root extract in a final volume of 15 μl. Reaction mixtures contained 0·5 U of *Taq* DNA polymerase (Promega), 0·2 μM of each forward and reverse primers, 100 μM of each dNTP and 1·5 mM of $MgCl_2$, in the recommended buffer conditions. The forward primer was labeled at the 5′ end with FAM. Cycling parameters were as follows: 95 °C for 2 min, followed by 30 cycles of 94 °C for 45 s, 60 °C for 45 s and 72 °C for 1 min, with a final extension step of 20 min at 72 °C. Amplification products were electrophoresed in an Applied Biosystems 377 DNA Sequencer™ and allele sizes were determined with Genescan Analysis software™.

Polymorphism: Table 1 shows the allele frequencies, observed heterozygosity, probability of exclusion[3] and polymorphism information content[4] obtained for 7 breeds of horses (*Equus caballus*).

Chromosomal location: The equine *CMA1* and *CKM* genes were, respectively, mapped to horse synteny groups UCD1 (ECA1) and UCD10 (ECA10)[2] by analysis of a horse-mouse somatic-cell hybrid panel[5–7].

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Table 1: Estimated allelic frequencies for equine microsatellites *CKM* and *CMA1*.

	CKM [a]Alleles									*CMA1* [a]Alleles						
	K	L	[b]M	N	*N*	*n*	H_o	PE	PIC	[c]M	N	*N*	*n*	H_o	PE	PIC
Horse breed																
Arabian	–	–	0·56	0·44	35	70	0·54	0·19	0·37	0·96	0·04	34	68	0·13	0·04	0·11
Andalusian	0·08	–	0·31	0·61	24	48	0·54	0·26	0·45	1·00	–	24	48	0·00	0	0
Morgan	–	–	0·59	0·41	23	46	0·39	0·18	0·37	0·89	0·11	24	48	0·22	0·09	0·18
Miniature Horse	–	0·06	0·40	0·54	24	48	0·67	0·20	0·46	0·94	0·06	24	48	0·13	0·05	0·11
Shire	–	–	0·92	0·08	24	48	0·17	0·07	0·14	0·98	0·02	24	48	0·04	0·02	0·04
Standardbred	–	–	0·77	0·23	24	48	0·29	0·15	0·29	0·90	0·10	24	48	0·13	0·08	0·16
Thoroughbred	–	–	0·33	0·67	35	70	0·37	0·17	0·34	1·00	–	32	64	0·00	0	0
Primers	F 5′-CCTCGGCCTTCTCACTTCTG									F 5′-TATGACCCCATGAGAAGCCAG						
	R 5′-GGGTTCCTGGGTCCAGTGTA									R 5′-GACCCCCTGAGTCTCCCTGT						
	GenBank acc. no. AF130753									GenBank acc. no. AF130752						

N, number of animals tested; *n*, number of alleles counted; H_o, observed heterozygosity; PE, probability of exclusion; and PIC, polymorphism information content. [a]Allelic types were assigned based on the PCR fragment size range observed, with [b]M = 113 bp and [c]M = 170 bp as the mid-values. The remaining alleles correspond to 2 bp increments or deductions. '-' indicates the allelic variant was not found among the animals tested.

The gene encoding a chicken chemokine with homology to human *SCYC1* maps to chromosome 1

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Source/description: PCR primers 1 and 2 were designed from the published sequence of a cDNA clone isolated from a spleen cDNA library which shows homology to the mammalian γ chemokine *SCYC1* (previously known as Lymphotactin or SCM-1)[1]. Comparison of the chicken sequence (EMBL accession number AF006742) with that of human *SCYC1* (EMBL accession number D43768) showed 55·8% sequence identity. This is a relatively high degree of sequence similarity for a comparison between a chicken and human immunological gene and suggests this clone may be the orthologue of mammalian *SCYC1*. This is supported by the pattern of tissues in which it is expressed[1].

Primer sequences: Primer sequences were derived from the chicken *SCYC1* cDNA sequence (EMBL accession number AF006742)

Primer 1: 5′-ACACAGAAAGTGGACATCAGAAGTATTGTCA-3′

Primer 2: 5′-TCTCTTCATAGCAGACTGCACCCAT-3′

PCR conditions: The reaction mixture comprised per reaction: primers at 5 μM, dNTPs each at 1 mM, 1·25 units of *Taq* polymerase, 200 ng genomic DNA, 1× PCR reaction buffer (Promega), 1·5 mM $MgCl_2$. The PCR profile was: denaturing; 94 °C for 60 s, annealing; 62 °C for 60 s, extension; 72 °C for 2 min. The reaction was carried out for 30 cycles with a final extension at 72 °C for 10 min using a Hybaid Omnigene Thermo-Cycler.

SSCP (single strand conformational polymorphism) analysis: An 873-bp PCR product was amplified from a genomic DNA template and its identity was confirmed by sequencing (EMBL accession number AJ242790). The PCR product was digested at 37 °C using a combination of *Rsa*I, *Hae*III and *Alu*I which gave products of 392-bp, 164-bp, 127-bp, 103-bp, 72-bp in size and several under 70-bp in size, corresponding to cleavage sites identifiable from the sequence. The digested product was ethanol precipitated, resuspended in loading buffer (98% formamide, 2% EDTA) and denatured at 90 °C for 5 min. Samples were placed on ice prior to analysis on a non-denaturing polyacrylamide SSCP gel (0·5X monomer solution of SEQUAGEL MD, National Diagnostics). The SSCP gel was run for 5 h at 8 watts at room temperature (25 °C), the gel was then silver stained using standard conditions[2].

Polymorphism: A polymorphism was observed between lines 6_1 and 7_2[3]. The segregation of bands was analysed in the Compton Marek's disease virus (MDV) mapping reference population3 (Fig. 1a). From sequence analysis of two of the progeny, representing the two different alleles, the line 6_1 allele shows base changes of C to T at position 1544, T to C at position 1668 and C to T at position 1920 when compared to the line 7_2 allele. These are located in the 127-bp, 72-bp and 392-bp fragments, respectively.

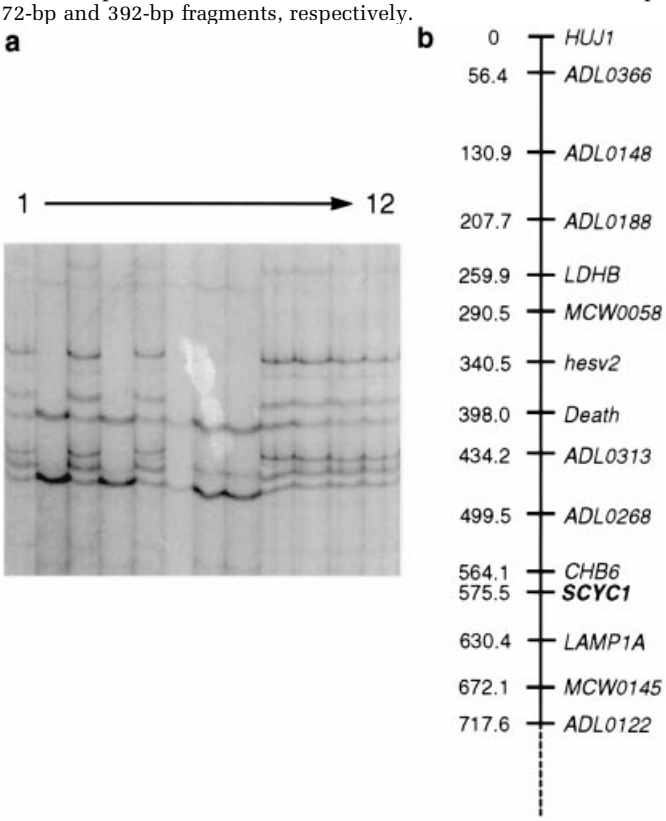

Fig. 1. (a) The SSCP pattern observed for offspring 1–12 from the backcross progeny of the MDV reference population are shown. The SSCP patterns observed in the ($6_1 \times 7_2$) F1 and 7_2 parents correspond to the patterns seen in offspring 1 and 2, respectively. (b) Linkage map of chicken chromosome 1 (distance in cM).

Mendelian inheritance: Autosomal codominance of the single stranded conformational polymorphism was observed in F1 and backcross matings between the Compton strains of chicken lines 61 and 7_2 (Fig. 1a).

Chromosomal location: The segregation pattern of the Line 61 SSCP in the 47 $(6_1 \times 7_2) \times 7_2$ backcross birds of the MDV reference population indicated that *SCYC1* is closely linked to *CHB6* (5 recombinants in 47 progeny, LOD 7·2) (Fig. 1b). SCYC1 is located on chromosome 1 in both human and mouse.

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Two polymorphic microsatellite markers from novel *Penaeus monodon* ESTs

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Source/Description: A lambda ZAP cDNA library was made from *Penaeus monodon* eyestalk mRNA[1]. cDNA was unidirectionally cloned into the *Eco*R1 and *Xho*1 polylinker of the ZAP Express vector (Stratagene). Recombinant plaques were sequenced at random and five plaques containing SSRs at the 5′ end were identified from 55 sequenced clones. We report the development of genetic markers from two of these microsatellite sequences.

Primer sequences:

PMCD001: 5′AGACAGTCAATCAGTCCGTCAG3′[1]
5′GCCATAAACTCTCTAACGACGTAG3′[2]

PMCD002: 5′CAGGGTGGCCATAAGAAATG3′[1]
5′CTCGACTGAAATTCTTCCTAGTTC3′[2]

PMCD001 is derived from EST AIMS-*P.mon*27 (Genbank accession no AI253824) and *PMCD002* from EST AIMS-*P.mon*35 (Genbank accession no AI253832)[1]. No homologues to these sequences were found using BLASTN searches[2] in the non-redundant Genbank and DBest databases.

Table 1. Characterisation of microsatellite markers

Micro-satellite marker	Repeat type	No. alleles	Size range of alleles (bp)	% Hetero-zygosity
PMCD001	$(AAT)_9AGT(AAT)_5$	7	196–213	73
PMCD002	$(TTTTC)_5(T)_{11}$	5	200–212	40

PCR conditions: PCR products were amplified in a 20-μl reaction volume using an MJ-Research Inc. PTC-100 Programmable Thermal Controller. PCR products were amplified using ≈ 5 ng of prawn DNA as template according to the conditions recommended for the T*th* Plus DNA polymerase (Fisher-Biotech International Ltd) and labelled with α^{32}P dCTP. The reaction mixture, including buffers and reagents (200 μM dNTPs; 1·5 mM $MgCl_2$; 0·3 μM each primer), DNA template and enzyme, was heated to 95 °C for 3 min followed by 30 cycles each consisting of 1 min at 95 °C, 2 min at 50 °C and 1 min at 72 °C. A final extension of 10 min at 72 °C was then carried out. Microsatellite amplification products were electrophoresed in 5% denaturing polyacrylamide gels. Radioactively labelled products were analysed by autoradiography.

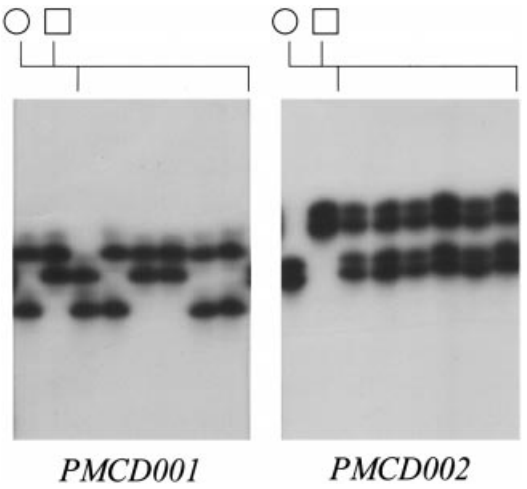

Fig. 1. Autoradiograph showing segregation of microsatellite alleles for *PMCD001* and *PMCD002* loci in the AIMS/CSIRO *Penaeus monodon* reference family no. 2 (http://shrimpmap.tag.csiro.au)

Heterozygosity: Heterozygosity was assessed by analysing these microsatellites on DNA from 32 unrelated *Penaeus monodon* sourced from the wild. Table 1

Mendelian inheritance: Co-dominant segregation of microsatellite alleles was confirmed by analysing segregation of these microsatellites in shrimp from the AIMS/CSIRO *Penaeus monodon* pedigreed reference families (http://shrimpmap.tag.csiro.au) Fig. 1.

Comment: Sequences amplified and were polymorphic in *P. esculentus*, *P. merguiensis* and *P. semisulcatus* using the *P. monodon* derived primers, making *PMCD001* and *PMCD002* useful type 1 markers for comparative mapping. Microsatellite markers from other less conserved areas of the genome not associated with genes are generally species specific[3].

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Chromosomal assignments and polymorphism information content in sheep for 12 cattle microsatellites

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Source/description: Sixteen sets of primers for cattle microsatellites were tested for their ability to amplify equivalent microsatellites in sheep. Eleven sets of primers amplified a single set of ovine bands, two amplified two or more sets of bands, and two amplified a monomorphic product (Table 1). These band sets all appeared to be microsatellites in that they consisted of a dominant band together with stutter bands. No ovine microsatellite products could be identified for *BL1023*. Multiple overlapping band sets that were too difficult to score were amplified by the *TGLA182* primers. The sizes of the different alleles were determined from comparisons with a sequencing ladder produced using the Forward M13 primer and pBSMB dsDNA template (Table 1).

Polymorphism/frequency: Amplified DNA was diluted 1:2 in formamide loading dye, heated at 95 °C for 3–5 min, then loaded onto a 6% denaturing polyacrylamide gel. The distribution of alleles for each microsatellite was determined for a population of unrelated sheep from nine different breeds (Merino, Border Leicester, Suffolk, Romney, Karakul, Finnish Landrace, Poll Dorset, Dorset and Carpet Master) and the Polymorphic Information Content (PIC) and heterozygosity values[1] are detailed in Table 1.

Mendelian inheritance and chromosomal location: Segregation was observed in nine, three-generation families comprising a total of 126 individuals belonging to the International Mapping Flock (IMF). The data were consistent with codominantly inherited alleles. Chromosomal assignments, with two-point LOD scores of greater than 3·0, were made for 12 loci (Table 1) and linkage results have been submitted to SheepBASE (http://zaphod1.agresearch.cri.nz:8002/). Based on similar product sizes and conserved chromosomal locations 11 of the primer sets (*BMS381*, *INRA129 PZ963*, *RM029*, *RM042*, *RM128*, *RM154*, *RM179*, *RM509*, *RME23*, and *RME25*) appear to amplify homologous loci in sheep and cattle. In contrast, the *HEL12* primers amplify loci that are not homologous in sheep and cattle.

PCR conditions: 20 ng of genomic DNA was amplified in a 5-μl reaction volume consisting of 67 mM Tris–HCl (pH 8·8), 1·5 mM $MgCl_2$, 16·6 mM $(NH_4)_2SO4$, 0·2 mg/ml gelatine, 0·45% Triton X-100, 100 μM dNTPs, 6·25 ng of both the forward and reverse primers, 0·125 Units of *AmpliTaq* DNA polymerase (Perkin Elmer), 0·275 μg of TaqStart Antibody (Clontech) and 0·25 μCi α^{33}P d-ATP (AMRAD). Reactions were performed in a 96 well plate and run on a DNA thermal cycler (PTC-100, MJ Research Inc), using the following conditions: one cycle of denaturation at 95 °C (2·5 min); 30 cycles of denaturation at 95 °C (30 s), annealing at the temperature indicated in Table 1 (30 s), extension 72 °C (30 s); and one cycle of extension 72 °C (2·5 min).

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Table 1 PCR conditions, number of alleles, polymorphic information content (PIC), heterozygosity and chromosomal locations of markers

Marker	Anneal temp (°C)	Number of alleles	PIC	Het	Null alleles	Size (bp)	Sheep chrom.	Cattle chrom.	Ref.
BM148	55	3	0·02	0·02		93–111	●		2
BMS381	51	7	0·76	0·80		109–123	2	8	3
BMS462	59	1				108†			4
BMS960	56	1				105†		X	4
HEL12†	54	7	0·57	0·64		171–189	4	20	5
INRA129§	56	4	0·52	0·58		156–160	2	8	6
PZ963	50/55	22††			Y	196–258	19	22	7††
RM029	54	8	0·67	0·72		84–102	3	5	8
RM042	51	3			Y	81–83	9	9	9
RM128	59	11	0.81	0.83		122–148	14	18	9
RM154	59	14	**	**		145–175	3	5	9
RM179	55	2	0·37	0·49		113–115	21	29	10
RM509	54	5	0·64	0·70		115–123	1	1	9
RME23	47	2	0·18	0·20		127–129	1	3	11
*RME25**	55	11			Y	125–157	7	10	11
TGLA182■	60					123–193			12

*Two sets of bands were amplified by the *RME25* primers.
†Sizing gels were not run for these microsatellites, however, coelectrophoresis of sheep and cattle samples revealed that sheep alleles were similar in size to cattle alleles.
†The *HEL12* primers amplified lots of background bands in sheep, many of which were more intense than the microsatellite that was scored.
§Single bp spacing.
●This marker was unable to be mapped as only one allele was found in the IMF.
**No PIC or heterozygosity values were determined for *RM154* as the presence of some alleles in a sample meant that other alleles would not amplify. Consequently typing was too difficult to do in the unrelated panel of sheep.
††Senese *et al.* (1998) reported 12 alleles and a PIC of 0·79.
■The *TGLA182* primers amplified at least two overlapping sets of bands with lots of stutter bands in both cattle and sheep samples.

University using NAGRP funds. This research was supported by a grant from Meat and Livestock Australia.

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Linkage mapping of goat ChirUCO, LSCV and SR-CRSP microsatellites in sheep

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Source/description: Sixty-three sets of primers for goat microsatellites were tested for their ability to amplify equivalent microsatellites in sheep. Thirty-six sets of primers amplified a single set of ovine bands, eight amplified two or more sets of bands, and ten amplified a monomorphic product (Table 1). These band sets all appeared to be microsatellites in that they consisted of a dominant band together with stutter bands. No ovine microsatellite products could be identified for *ChirUCO3*, *LSCV04*, *LSCV13*, *LSCV17*, *LSCV20*, *LSCV23*, *LSCV46*, *SR-CRSP-11* and *SR-CRSP-22*. Multiple overlapping band sets that were too difficult to score were amplified by the *SR-CRSP-23* primers. The sizes of the different alleles were determined from comparisons with a sequencing ladder produced using the Forward M13 primer and pBSMB dsDNA template (Table 1).

Polymorphism/frequency: Amplified DNA was diluted 1:2 in formamide loading dye, heated at 95 °C for 3–5 min, then loaded onto a 6% denaturing polyacrylamide gel. The distribution of alleles for each microsatellite was determined for a population of unrelated sheep from nine different breeds (Merino, Border Leicester, Suffolk, Romney, Karakul, Finnish Landrace, Poll Dorset, Dorset and Carpet Master) and the Polymorphic Information Content (PIC) and heterozygosity values[1] are detailed in Table 1.

Mendelian inheritance and chromosomal location: Segregation was observed in nine, three-generation families comprising a total of 126 individuals belonging to the International Mapping Flock (IMF). The data were consistent with codominantly inherited alleles. Chromosomal assignments, with two-point LOD scores of greater than 3·0, were made for 42 loci (Table 1) and linkage results have been submitted to SheepBASE (http://zaphod1.agresearch.cri.nz:8002/). Based on similar product sizes and conserved chromosomal locations, 28 of the primer sets (*LSCV03*, *LSCV05*, *LSCV06*, *LSCV08*, *LSCV10*, *LSCV11*, *LSCV14*, *LSCV15*, *LSCV21*, *LSCV22*, *LSCV24*, *LSCV28*,*LSCV29*, *LSCV30*, *LSCV32*, *LSCV33*, *LSCV36*, *LSCV37*, *LSCV40*, *LSCV41*, *LSCV42*, *LSCV44*, *LSCV52*, *LSCV55*, *LSCV105*, *SR-CRSP-5*, *SR-CRSP-6*, and *SR-CRSP-10*) appear to amplify homologous loci in sheep and goats. In contrast, the *LSCV12* primers amplify loci that are not homologous in sheep and goats, and no homologue of the higher molecular weight set of *LSCV10* bands (*LSCV10T*) has been described for goats. As no goat chromosomal information is available for the remaining loci that have been mapped in sheep, it is not possible to determine whether the loci amplified by these primers are homologous in sheep and goats. However, given that similar sized products are amplified for both species it is likely that the majority of these loci will be homologous for sheep and goats.

PCR conditions: 20 ng of genomic DNA was amplified in a 5-μl reaction volume consisting of 67 mM Tris–HCl (pH 8·8), 16·6 mM $(NH_4)_2SO4$, 0·2 mg/ml gelatine, 0·45% Triton X-100, 1·5 mM or 2·0 $MgCl_2$ (see Table 1), 100 μM dNTPs, 6·25 ng of both the forward and reverse primers, 0·125 Units of *AmpliTaq* DNA polymerase (Perkin Elmer), 0·275 μg of TaqStart Antibody (Clontech) and 0·25 μCi α^{33}P d-ATP (AMRAD). Reactions were performed in a 96 well plate and run on a DNA thermal cycler (PTC-100, MJ Research Inc), using the following conditions: one cycle of denaturation at 95 °C (2·5 min); 30 cycles of denaturation at 95 °C (30 s), annealing at the temperature indicated in Table 1 (30 s), extension 72 °C (30 s); and one cycle of extension 72 °C (2·5 min).

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Ten equine dinucleotide microsatellite repeats *HTG18-19*, *HTG22-24*, *HTG26-27*, *HGT29-30* and *HTG32*

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Source/description: An equine size fractionated (200–600 bp) pUC18 plasmid genomic library was screened with radioactively labelled oligonucleotides $(CT)_{15}$ and $(CA)_{15}$ (Pharmacia) as probes. Positive clones were sequenced on both strands using an Applied Biosystems ABI377 sequencer. Primers were designed in repeat flanking regions and used in microsatellite PCR amplification. Primer sequences are given in Table 1.

PCR conditions: Amplifications was performed in a MJ Research (PTC-100) thermal cycler in 10 μl reactions containing 0·25 U *AmpliTaq* DNA polymerase (Perkin Elmer), 200 μM dNTPs, 1·5 mM $MgCl_2$ (except for markers *HTG22* and *HTG26* for which 2·0 mM $MgCl_2$ was used), 50 mM KCl, 10 mM Tris–HCl pH 8·3, 0·001% (w/v) gelatin, 50 ng genomic DNA and 2 pmol of each primer. The PCR profile consisted of one cycle of 94 °C for 3 min, 65 °C (*HTG23*, *HTG27* and *HTG30*)/ 62 °C (*HTG19* and *HTG29*)/ 60 °C (*HTG18*, *HTG22*, *HTG26* and *HTG32*) for 30 s and 72 °C for 1 min, followed by 29 cycles of 94 °C for 30 s, 60 °C (*HTG23*, *HTG27* and *HTG30*)/ 57 °C (*HTG19* and *HTG29*)/55 °C (*HTG18*, *HTG22*, *HTG26* and *HTG32*) for 30 s and 72 °C for 30 s. After the final cycle a prolonged extension step of 10 min was included. For marker *HTG24* a touchdown PCR profile was used. The annealing temperature ranged from 70 to 60 °C with a decrease of 0·5 °C/cycle for 20 cycles followed by a constant annealing temperature of 60 °C for ten cycles. The PCR products were mixed with loading buffer (95% formamide, 5% xylene cyanol, 5% bromophenol blue, 10 mM NaOH) and separated by electrophoresis for 1–2 h on 6% denaturating polyacrylamide gels (SequagelXR, National Diagnostics, Atlanta, GA). Detection of DNA fragments was made using silver staining (Promega Corporation, Geneprint STR Systems Manual, Part no. TMD004).

Polymorphism: Polymorphism screening was done in 20 unrelated horses, including five Gotland ponnies, five Shetland ponnies, five Swedish Ardennes and five Swedish coldbred horses. Observed heterozygosities varied between 0·35 and 1·00 in this material.

Chromosomal location: Unknown

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Table 1. Characterization of ten horse microsatellites

Locus	Repeat sequence	No. of alleles	Size (bp)	Observed heterozygosity	Primer sequences
HTG18	$(CA)_{11}$	6	159*	1·00	CTGAAACCTCATTTTATACAG
					TACTAGAACACAGAAAGCCTA
HTG19	$(CA)_{13}$	4	130–144†	0·40	GTATGTGCTGTACCTTCTGC
					ATGAGAAAGACGATAGATGATAT
HTG22	$(CT)_8GTTT(CT)_9(CA)_{14}$	4	188*	0·50	TACTACATTTCATCCTCCACAT
					GGAGAACTTCCCAGAGAGCA
HTG23	$(CA)_{19}$	5	191–199†	0·35	GTCCTTCAGAGTTGTCCCTG
					GGAGAACAACCTTGCCTGAG
HTG24	$(CA)_{14}$	5	174*	0·65	CTCAGGGCTAATCTTCCTCA
					TGTAAATTCTCCTATGGAAGCA
HTG26	$(CA)_{12}(AT)_{10}$	7	141*	0·35	CCTGGAAACCATCATTCTACT
					CTCCAGGCTGAGTTATTTGTA
HTG27	$(CT)_{13}GA(CT)_3$	7	161*	0·35	ATATGTCATATTTGAACAAGTCG
					GCACTGAAATCGAACATCTAA
HTG29	$(CA)_5CGCATA(CA)_2T(CA)_{18}$	5	119–129†	0·65	CTATTTCCAGTCTTTGTGTGT
					CCATAATAAAACATTAAAGATCAG
HTG30	$(CA)_{19}$	5	242*	0·70	TCAAGGCAAATCTTTCCCAG
					GTAAAATAACAAGTTGTTCCAG
HTG32	$(CA)_3CT(CA)_2C(CA)_{15}$	5	150–160†	0·35	CCTGAAACCTCAGTAAACAGA
					TGTGGCTTTGGTGTGGAAAC

*Size of the cloned allele.
†Estimated size range.

A *Taq*I PCR-RFLP at the bovine myogenic factor (*MYF5*) gene

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Source/description: A whole genome scan indicates that the proximal region of BTA5 might contain genes influencing the susceptibility to develop atresia coli in cattle[1]. To increase marker density in this region a PCR-RFLP was developed in the myogenic factor 5 (*MYF5*) gene. Six pairs of primers were designed from the bovine sequence (GenBank accession number M95684) to amplify intron 1, intron 2, and the 3′ UTR of the *MYF5* gene[2]. DNA samples of ten unrelated German Holstein sires were successfully amplified yielding single bands of predicted sizes. The PCR-products were digested with restriction enzymes *Taq*I, *Pvu*II, *Bst*EII, and *Hind*III followed by agarose gel electrophoresis. DNA was visualised by a UV light source after staining with ethidium bromide. The 445-bp PCR-product of intron 2 (primers MYFi2F/ MYFi2R) showed a polymorphic recognition site for *Taq*I. Direct sequencing of the products from three animals with different genotypes with IRD800 labelled standard universal sequencing primer showed complete identity to the published sequence. At nucleotide position 1948 of the *MYF5* gene (GenBank accession number M95684) an A(→)G mutation creates a *Taq*I restriction site. The sequencing reactions were separated on 0.2 mm 6% polyacrylamide sequencing gels using a LI-COR 4200 automated sequencer.

Primer sequences:
Forward primer MYFi2F: 5′ ACA GCG TCT ACT GTC CTG ATG 3′

Reverse primer MYFi2R: 5′ CGT GGC ATA TAC TAA GGA CAC 3′

PCR conditions and analysis: A PCR amplification (20 μl final volume) was performed using 20 ng of genomic bovine DNA, 1x PCR buffer (Promega), 1.5 mM $MgCl_2$, 100 μM each dNTP, 4 pmol each primer, and 1 U *Taq* polymerase (Promega). The thermocycler profile was 94 °C for 4 min; 38 cycles of 94 °C for 30 s, 58 °C for 60 s, and 72 °C for 60 s; followed by a final extension at 72 °C for 4 min.

Polymorphism: Ten microlitres of PCR-product was digested with 2.5 U *Taq*I (N.E.B.) and separated on a 2% agarose gel. Three genotypes after the *Taq*I digestion of the 445-bp fragment are shown in Fig. 1. The mutation introduces a *Taq*I restriction site generating two bands of 352 bp and 93 bp (Allele *2*). Allele frequencies were estimated by typing the International Bovine Reference Panel (IBRP)[3] parents (1 = 0.30 and *2* = 0.70) and 48 unrelated German Holstein cattle (1 = 0.32 and 2 = 0.68).

Mendelian inheritance: The animals of the IBRP were genotyped and the two alleles segregated in a pattern consistent with Mendelian inheritance.

Chromosomal location: MYF5 was physically assigned to bovine chromosome 5 q13[4] and RFLPs have been described for linkage mapping by Southern analysis[5]. Previous linkage mapping has placed *MYF5* at 0 cM from microsatellite marker *BPI*[6]. After genotyping the IBRP the results of the two point linkage analysis with the markers of the Cattle Genotypic Database confirm the location of the *MYF5* gene on the proximal region of BTA5 (Tab. 1; W. Barendse, personal communication). Compared to the USDA linkage map[6] *MYF5* is placed further proximal and close to *SYT1*. The results improve a link between the genetic maps in this region.

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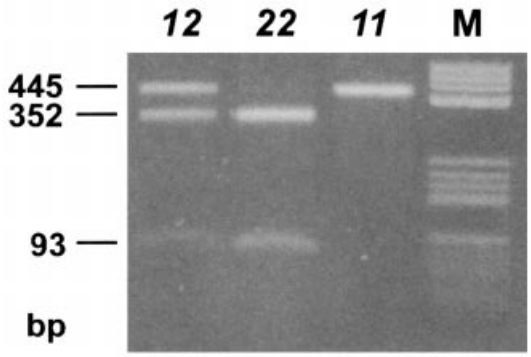

Fig. 1. *Taq*I polymorphism in the *MYF5* gene. One animal of each genotype

Table 1. Two point linkage results of *MYF5-Taq*I with closely linked loci

Loci	θ	lods	θ_f	θ_m	lods
*MYF5-Taq*I–*BP1*	0.12	13.24	0.11	0.14	13.31
*MYF5-Taq*I–*SYT1*	0.03	16.41	0.00	0.03	16.69
*MYF5-Taq*I–*BM6026*	0.09	13.43	0.14	0.07	13.57

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The porcine adenosine monophosphate deaminase 1 (*AMPD1*) gene maps to chromosome 4

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Source/description: Adenosine monophosphate deaminase (AMP deaminase; AMPD; EC 3·5·4·6) catalyzes the deamination of AMP to IMP in skeletal muscle and plays an important role in the purine nucleotide cycle. AMPD is a complex allosteric enzyme encoded by a multigene family in mammals. The *AMPD1* gene is expressed predominantly in skeletal muscles (M isoform), the *AMPD2* gene encodes the L (liver) isoform, and the *AMPD3* gene encodes two erythrocyte isoforms, E1 and E2. Both the *AMPD1* and *AMPD2* genes are located on human chromosome 1p21-p13, and *AMPD3* is situated on human chromosome 11pter-p13[1]. To study the porcine *AMPD1* gene we designed degenerate PCR primers (Pair 1) using human and rat sequences[2] (EMBL accession numbers M98818 and M98819). The sequence of the porcine PCR fragment was determined and used to design a new pair of PCR primers (Pair 2) that was used to search for polymorphism.

Primers:

Pair 1: Forward 5′ CCGSCGYCTGAAGTTCCTCTC 3′

Reverse 5′ CTGGTTCATGCARGCRGCTGC 3′

(S stands for G or C, Y for C or T, and R for A or G)

Pair 2: Forward 5′ GAAGAACAATCCTCACCGGGACTT 3′

Reverse 5′ GGCTGCGTGGATATGGGTGTC 3′

PCR conditions/cloning/sequencing: PCR, using Primer Pair 1, was carried out in 50 μL containing 100 ng porcine genomic DNA, reaction buffer, 1·5 mM $MgCl_2$, 200 μM each dNTP, 10 pmol each primer and 2·0 U of a mixture of Deep Vent (New England Biolabs, Schwalbach/Taunus, Germany) and *Taq* polymerases. Amplification conditions were 2 min at 95 °C followed by 34 cycles of 95 °C (45 s), 58 °C (1 min) and 72 °C (1·5 min), with a final extension at 72 °C (7 min). A single weak zone (≈ 1·4 kb) was observed on agarose gel electrophoresis. The fragments from Pietrain and Meishan were subcloned in pUC18 and sequenced (ALFexpress Sequencing System, Pharmacia Biotech, Uppsala, Sweden). The sequence was homologous to parts of exons 7 and 8 and all intervening intron of the human *AMPD1* gene (EMBL M98818). The porcine sequence has been deposited in the EMBL database under accession number AJ242995.

PCR, using Primer Pair 2, was performed in 25 μL using 100 ng genomic DNA, standard PCR buffer, 2·0 mM $MgCl_2$, 200 μM each dNTP, 10 pmol each primer and 0·5 U *Taq* polymerase. Amplification conditions were 2 min at 95°C, followed by 30 cycles of 95°C (30 s), 58°C (45 s) and 72°C (1·5 min), with a final extension at 72°C (7 min). Sequencing of the cloned PCR fragment (≈ 1·3 kb) confirmed that it was *AMPD1*.

Polymorphism/Mendelian inheritance/allele frequencies: Polymorphism was found after restriction with *Rsa*I (Fig. 1). Two alleles were observed (exact sizes of fragments were determined according to the EMBL AJ242995 sequence): Allele *A* - fragments 882 + 159 + 77 + 73 + 46 + 32 bp. In allele *C* the 882 bp fragment was cut to fragments of 631 and 251 bp. The two alleles differ at position 426 of EMBL AJ242995 sequence (A—C transition). This site is within an intron. Codominant inheritance was demonstrated in the Hohenheim Meishan × Pietrain (M × P) and Wild Boar × Meishan (W × M) three-generation pedigrees[3]. Both alleles were present in Meishan (n = 12; frequency of *A* - 0·33 and *C* - 0·67). Other breeds tested (Large White, 25; Landrace, 24; Pietrain, 18; Hampshire, 6; Black Pied Prestice, 7; Czech Meat Pig, 14; Duroc, 14) were monomorphic for allele *A* except for one Large White pig, which was heterozygote *AC*.

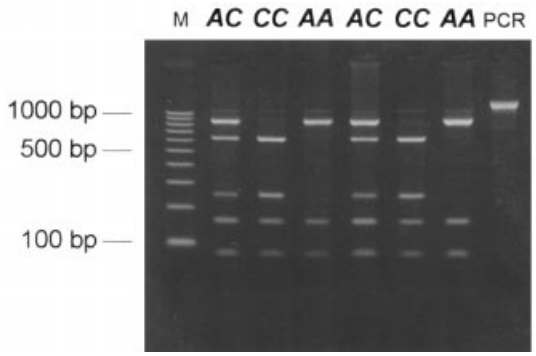

Fig. 1. Agarose gel (2·5%) showing genotypes of porcine *AMPD1* following digestion of the PCR products with *Rsa*I. The genotypes (*AA AC CC*) are shown at the top. M, 1000-100 bp ladder; PCR, undigested PCR fragment.

Linkage mapping/association studies: Linkage mapping of *AMPD1* was performed using the CRI-MAP package[4] in the Hohenheim M × P and W × M pedigrees[3]. *AMPD1* was found to be tightly linked with the *NGFB* gene that was previously mapped by Kopečný *et al.* (2000)[5] to a chromosome 4 linkage group *S0073-EAL-NGFB-Sw2435*. The distances (Kosambi cM; sex average) in M × P pedigree are as follows: *S0073*–16·2-*EAL*-4·6-*NGFB*-0·0-**AMPD1-**11·6-*Sw2435*. The porcine *NGFB* was earlier assigned to chromosome 4q1·6-q2·3[6], and this should also be the location of *AMPD1*. The porcine *AMPD1* maps within known QTL with effects on carcass traits (carcass weight cold, shoulder meat weight, bacon meat weight, chops weight, head weight and muscle area) in the Hohenheim W × P and

M × P QTL populations[7,8]. *AMPD1* may be regarded a positional candidate for these traits.The *AMPD1* PCR-RFLP is fixed for different alleles in the founders of the M × P pedigree (*C* in Meishan, *A* in Pietrain), and associations of the alleles with the carcass traits were observed in F2. However, tests for linkage disequilibrium in other populations are required in order to examine *AMPD1* as a candidate gene for these traits.

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Dinucleotide repeat polymorphism at the ovine M*cMA1*, M*cMA2*, M*cMA5*, M*cMA8*, M*cMA9*, M*cMA11*, M*cMA14*, M*cMA20*, M*cMA24*, M*cMA26* loci

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Source/description: After digestion with *Sau*3A a size selected (200–500 bp) ovine genomic DNA library was constructed in pBluescript SK+ phagemid vector and screened with a radioactively labelled $(GT)_8$ oligonucleotide. Oligonucleotide primers were designed using the computer program PRIMER (The Whitehead Institute for Biomedical Research, MA, USA) to minimise self-annealing and to achieve a Tm of ≈ 62 °C. The microsatellites were amplified by PCR, and analysed by denaturing polyacrylamide gel electrophoresis.

Primer sequences:

McMA1 (forward primer): CATTACAgCCTgTgTgAgTgTg
(reverse primer): gATAgTTCTATCCAACCgTCCC

McMA2 (forward primer): TCACCCAACAATCATgAAAC
(reverse primer): TTAAATCgAgTgTgAATggg

McMA5 (forward primer): TTAAgAATTCTCTCTCCCTCCC
(reverse primer): TgACCTCATACAgATTgATgTTg

McMA8 (forward primer): TATTgACATTTTTTATTCATTTgC
(reverse primer): AggAATTCCCTTTTTgATgg

McMA9 (forward primer): AgTCCAgAAACAAgTCTACCCg
(reverse primer): GCCATAgTCCATgCggTC

McMA11 (forward primer): TCTCTggCACCTTAggTTgC
(reverse primer): CgACTCTAgAggATCATgAATAgC

McMA14 (forward primer): TgTTTCCTCTTCTCCAAATATC
(reverse primer): gCCCTATTAAgCCAATATACAg

McMA20 (forward primer): TTggAggTgCTAgAggTgTAg
(reverse primer): CTAgAATgACAgTTCCAACTgg

McMA24 (forward primer): TgACCTCAgggCCTATTCC
(reverse primer): gATCCCAgTTACTCCTCACAgg

McMA26 (forward primer): TCTCTgCTTTCCAgCCTTATTC
(reverse primer): AgAgCTTTTAggACAgCCACC

Guanine nucleotides in the primer sequence are represented by lower case 'g' to ensure that they can be distinguished from cytosine 'C'. All primer sequences are listed 5′ to 3′. Limited amounts of these primers are available from the authors' laboratories.

Table 1. Polymorphic information content (PIC), heterozygosity, and allele frequencies for M*cMA* microsatellites

	McMA1	McMA2	McMA5	McMA8	McMA9	McMA11	McMA14	McMA20	McMA24	McMA26
Repeat sequence	$(AT)_9GT(AT)_7(GT)_{13}$	$(AC)_{28}$	$(AC)_{15}$	$(AC)_{18}$	$GT_{19}CTAT(GT)_5$	$(GT)_{13}$	$(AC)_{17}$	$(AC)_{22}$	$(AC)_{14}$	$(GT)_{31}$
GenBank accession number	AF098772	AF098773	AF098774	AF098775	AF098776	AF098957	AF098958	AF098959	AF098960	AF098961
PIC	0·87	0·89	0·73	0·65	0·56	0·72	0·67	0·73	0·44	0·86
Heterozygosity	0·89	0·90	0·77	0·69	0·60	0·77	0·72	0·76	0·49	0·88
Number of unrelated sheep	49	47	49	49	48	49	49	47	49	48
Ovine chromosomal location†	23	13	8	1	6	10	6	17	11	18
Allele size (frequency)	146 (0.03)	201 (0.02)	162 (0.10)	164 (0.01)	142 (0.01)	222 (0.02)	216 (0.02)	129 (0.01)	194 (0.08)	212*
	144 (0.10)	197 (0.12)	160 (0.26)	161 (0.01)	132 (0.05)	220 (0.04)	214 (0.39)	127*	193 (0.01)	210 (0.03)
	142 (0.02)	195 (0.01)	158 (0.06)	158 (0.06)	130 (0.03)	218 (0.04)	204 (0.04)	125 (0.13)	191 (0.19)	208 (0.4)
	140 (0.06)	193*	156 (0.01)	156 (0.01)	128 (0.16)	212 (0.01)	202 (0.02)	123 (0.44)	189 (0.68)	206 (0.08)
	138 (0.04)	191 (0.10)	154 (0.05)	155 (0.01)	126 (0.01)	196 (0.02)	200 (0.33)	121 (0.03)	179 (0.03)	204 (0.02)
	136 (0.23)	189 (0.09)	152*	154 (0.15)	124*	194 (0.02)	198 (0.14)	119 (0.02)		203 (0.01)
	134 (0.10)	187*	150 (0.37)	152 (0.07)	122 (0.59)	192 (0.01)	196 (0.06)	117 (0.03)		202 (0.10)
	132 (0.06)	185 (0.02)	148 (0.15)	151 (0.01)	120 (0.15)	190 (0.01)		107*		201 (0.01)
	130 (0.11)	183 (0.12)		150 (0.13)		186 (0.34)		101 (0.15)		200 (0.01)
	128 (0.08)	181 (0.02)		139 (0.52)		182 (0.17)		99 (0.10)		198 (0.10)
	126 (0.10)	179 (0.11)		137 (0.01)		180 (0.31)		93 (0.10)		196 (0.04)
	124 (0.05)	177 (0.06)				176 (0.01)				195*
		173 (0.01)								194 (0.17)
		165 (0.07)								192 (0.02)
		163 (0.02)								190 (0.02)
		161 (0.19)								189 (0.08)
		157 (0.04)								188 (0.25)

*Alleles found in sheep that were not part of the unrelated panel.
†Chromosomal location was determined by linkage analysis using the IMF (pers. comm. A. Crawford).

Mendelian inheritance: Segregation was observed in nine, three-generation families comprising a total of 126 individuals (International Mapping Flock). The data was consistent with codominantly inherited alleles. Alleles were designated according to the number of base pairs detected in the fragment after PCR amplification. A sequencing ladder using the Forward M13 primer, pBSMB dsDNA template was used to determine the size of the different alleles (Table 1). Two point linkage results for the *McMA* microsatellites have been submitted to SheepBASE.

Frequency: The distribution of alleles for each microsatellite was determined for a population of unrelated sheep from nine different breeds (Merino, Border Leicester, Suffolk, Romney, Karakul, Finnish Landrace, Poll Dorset, Dorset, Carpet Master). The polymorphic information content (PIC), heterozygosity and chromosomal location are detailed in Table 1. Amplification was attempted for two cattle genomic DNA samples and products were obtained for *McMA1* (1 band), *McMA2* (1 band), *McMA5* (1 band), *McMA9* (2 bands), *McMA14* (1 band), *McMA20* (1 band), *McMA24* (1 band) and *McMA26* (1 band).

PCR conditions: 40 ng of genomic DNA was amplified in a 10 μL reaction volume consisting of 67 mM Tris-HCl (pH 8·8), 16·6 mM $(NH_4)_2SO4$, 0·2 mg mL^{-1} gelatine, 0·45% Triton X-100, 1·5 mM $MgCl_2$, 100 μM dNTPs, 12·5 ng of both the forward, reverse primers, 0. 25 Units of AmpliTaq DNA polymerase (Perkin Elmer), 0·55 μg of Taq Start Antibody (Clontech) and 0·5 μCi α^{33}P d-ATP (AMRAD). Reactions were set up in a 96-well plate and run on a DNA thermal cycler (PTC-100, MJ Research Inc), using the following conditions: one cycle of denaturation at 95 °C (2·5 min); 29 cycles of denaturation at 95 °C (30 s), annealing 52 °C (30 s), extension 72 °C (30 s) and one cycle of extension 72 °C (2·5 min).

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The isolation and characterization of 18 equine microsatellite loci, *TKY272–TKY289*

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Table 1. Characterization of equine microsatellite loci, *TKY272–TKY289*

Locus accession no.	Repeat structure product size	Alleles	Size range (bp)	HTZ	PIC	PE	Primer sequence 5′–3′
TKY272	(CA)21	3	100–116	0·573	0·480	0·278	CTTGAAGATTCAGGTCATGG
AB033923	116 bp						ATATCATAGCAAATGGCAGG
TKY273	(CA)22	7	174–194	0·719	0·671	0·476	GATCACTGGCGAGGGTAAGC
AB033924	192 bp						TATGTTCCCGATTCGCAAGC
TKY274	(CA)24	4	112–132	0·502	0·465	0·289	CATTCTGGAATTGAAACCTC
AB033925	130 bp						GGGAGTTCTGTTCTGAATGG
TKY275	(CA)15	4	144–154	0·489	0·451	0·278	TCTCAGTGGATATAACTAGC
AB033926	144 bp						GAGATGGATACAGATAGAAG
TKY276	(AC)13	4	87–101	0·549	0·461	0·268	TTAGCACTTTATCTCCCTGC
AB033927	97 bp						TGTGTCTGCAATTTACTCTG
TKY277	(CA)21	2	88–90	0·490	0·370	0·185	TTCCAAGTTTACCGGATGAG
AB033928	88 bp						TGCAATGAAAGTACATAGTATG
TKY278	(AC)24	5	104–132	0·699	0·644	0·446	CTAGGGAATACAGAGAGAGC
AB033929	132 bp						GCCACTCCGGTAACAAAATC
TKY279	(CA)18	6	119–133	0·733	0·693	0·510	AATGAATGAGACTTGAACCC
AB033930	127 bp						TCTGCTGTTTTAGGCTCGG
TKY280	(CA)17...(CA)10...	4	309–321	0·319	0·284	0·153	GAGGAGACCAAAATAACAGG
AB033931	...(CA)7...(CA)9 313 bp						ACTCCCTGCTTTGCACTCTG
TKY281	(CA)12CT(CA)6	2	178–194	0·157	0·144	0·072	GCTTGGACAAGCGAATAATGAC
AB033932	194 bp						GATCAGTCACTGCCAGTGG
TKY282	(CA)18	5	147–155	0·592	0·508	0·310	TTACATCTCTGCACTCTCCC
AB033933	147 bp						GGTCTCATGCAAAACGCAGG
TKY283	(CA)15TA(CA)8	3	203–207	0·359	0·329	0·186	GGAAGTCATCTCTTTCCAGC
AB033934	203 bp						AATACATGATTCCTGCCTGC
TKY284	(CA)18	6	159–175	0·732	0·685	0·492	CTGGACTAGAGTCAGATTGC
AB033935	163 bp						AACAGGATTCCCCCAATGCC
TKY285	(CA)22	4	164–174	0·644	0·577	0·371	ACCAATGGTAAACATGGCAG
AB033936	172 bp						CTGAAGAGGCAAGGAAAAGG
TKY286	(CA)13	4	94–112	0·609	0·543	0·341	TCCTTAACAACAAAGACACC
AB033937	104 bp						ATGGGCATATTAAGATGCAC
TKY287	(CA)17...(CA)12	7	224–240	0·806	0·780	0·619	ATCAGAGAACACCAAGAAGG
AB033938	230 bp						TCTCTGCTATAGGTAAGGTC
TKY288	(AC)18	4	139–157	0·234	0·223	0·123	AGTATGACAGCCCACTCTCG
AB033939	155 bp						ATGCATTTCAAGGAAGCCAG
TKY289	(CA)9	1	151	0	0	0	CATTATTCACAATAGCCAGG
AB033940	151 bp						TCCATGTTGTTGCAAATGGG

The nucleotide sequence data reported in this paper will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession numbers AB033923, AB033924, AB033925, AB033926, AB033927, AB033928, AB033929, AB033930, AB033931, AB033932, AB033933, AB033934, AB033935, AB033936, AB033937, AB033938, AB033939, AB033940.

Source/description: Microsatellites were isolated from a microsatellite enrichment-library (Tozaki *et al.* unpublished). Microsatellite enrichment library was performed as following procedures[1]. Briefly, horse genomic DNA was prepared from one stallion as the source. DNA was digested with restriction enzyme *Sa*u3AI, and compatible oligonucleotide adapters were ligated to the restricted fragments. After the ligation, PCR was performed using the adapter sequences to obtain the source DNA for a streptavidin-biotin capture method. Primer extension was performed by using a biotinylated oligo-(CA)8, and dCTP, dATP as a substrate. dCTP, dATP were incorporated only to repeat regions. The mixtures were then incubated with streptavidin-coated magnetic beads, and the magnetic bead-complexes were captured by a magnet. Single-stranded DNAs containing microsatellites were released from the beads, and double-stranded (ds) DNAs prepared by PCR using the adapter sequences. Finally, the ds DNAs enriched for (CA)*n* repeats were inserted into a T-vector (Promega). These recombinants were transformed into competent XL-1 Blue MRF′ *E. coli* cells by electroporation (Bio-Rad). The resulting clones were sequenced using automated cycle sequencing by an automatic DNA Sequencer (A.L.F. express DNA Sequencer, Amersham Pharmacia Biotech). About 95% of the sequenced clones were positive for (CA)*n* repeats. In addition, about 90% of these clones had over 12-repeats, thus are more likely to be polymorphic[2].

PCR conditions: PCR was performed in a total volume of 20 μL of the following mixture: 20 ng of horse genomic DNA; 5 pmol of each primer, 200 μM of dNTPs, 2 μl of 10 × reaction buffer; and 0·1 unit of *Taq* polymerase (Takara, Japan). PCR amplification entailed initial denaturation (94, 4 min); 30 cycles of 1 min each at 94, 55, 72 °C, then 10 min at 72 °C for final extension in a GeneAmp PCR System 9600 (Perkin-Elmer Cetus).

Polymorphism: The amplified fragments were analyzed by electrophoresis with an automatic DNA Sequencer (A.L.F. express DNA Sequencer, Amersham Pharmacia Biotech). HTZ (Heterozygosity), PIC (Polymorphism Information Content), and PE (probability exclusion) values were determined using DNA from Thoroughbred horses (n = 35~40). See Table 1 for results.

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A *Nci*I PCR-RFLP within intron 2 of the porcine insulin-like growth factor 2 (*IGF2*) gene

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Source and description: Insulin-like growth factor 2 (IGF2, somatomedin A) is a member of insulin/igf/relaxin growth factor family and possesses growth-promoting activity. A single nucleotide polymorphism (G → A transition) in exon 2 and a microsatellite (SW9) located 800 bp downstream of the *IGF2* stop codon have been mapped by linkage analyses[1,2]. These two polymorphic markers have been used to map the *IGF2* gene to the proximal end of chromosome 2. A QTL, which exhibits imprinting such that only the paternal allele is expressed, and which influences muscle mass, has been mapped close to the *IGF2* locus[1,2]. Porcine mRNA of the *IGF2* gene was sequenced by Catchpole *et al.* [3] (EMBL accession number X56094). From this sequence, compared for exon-intron structure with the DNA sequence of human *IGF2*[4] (EMBL accession number X03562), PCR primers (forward, reverse A) were designed to give an amplification product encompassing a part of exon 2, exon 3 and intervening intron 2 (by analogy with the human ortholog). The PCR product of expected length approximately 2 kb was observed on an agarose gel and its identity was verified by sequencing. From provisional terminal sequence of this amplimer, in intron sequence 434–456 bp from 3′ end of the PCR product, the reverse primer B was designed. Reverse primer B together with forward primer gave an amplimer about 1. 6 kb, which is a more convenient for testing. Identity of this PCR product was again confirmed by provisional terminal sequencing.

Primer sequences:

Forward: 5′ AGACTCTGTGCGGCGGGGAGCT 3′

Reverse A: 5′ CAGCAGGGCCAGGTCGCAGCTA 3′

Reverse B: 5′ CGA GTG CGG TCC CCA ATG GAT 3′

PCR conditions: PCR was performed in 50 μl reactions using 100 ng porcine genomic DNA, 0·2 μM each primer, 200 μM each dNTP, 2.0 mM $MgCl_2$, 1·5 unit of *Taq* polymerase in standard PCR buffer. After an initial 95 °C denaturation step (2 min) the PCR was carried out at 95 °C (30 s), 70 °C (30 s), 72 °C (50–110 s, extension 2 s for each cycle, the last extension 7 min) for 30 cycles. Digestion of 10 μl of each PCR product was performed with 3 units of *Nci*I at 37 °C overnight.

Polymorphism: Polymorphism was found with *Nci*I. This restriction enzyme cuts the 1·6 kb amplimer into several fragments (Fig. 1). Allele *A*, in which the polymorphic restriction site is absent, is characterised by the presence of the largest fragment of approximate length 0.9 kb, while for allele *B* which possesses the polymorphic restriction site this fragment is cut to yield a fragment of about 0·8 kb and a short one barely detectable on the gel.

Mendelian inheritance/allele frequencies: Genetic analysis performed in three two-generation families from the Hohenheim PiGMaP pedigree[3] showed codominant inheritance of the two alleles. An example of segregation in a single family is shown in Fig. 1. Allele frequencies of *IGF2* gene in unrelated animals of four pig breeds are given in Table 1.

Table 1. Allele frequencies of *IGF2* gene in different pig breeds

Breed	Number of animals	Allele A	Allele B
Landrace	12	0·42	0·58
Large White	21	0·17	0·83
Duroc	12	0·21	0·79
Pietrain	12	0·00	1·00

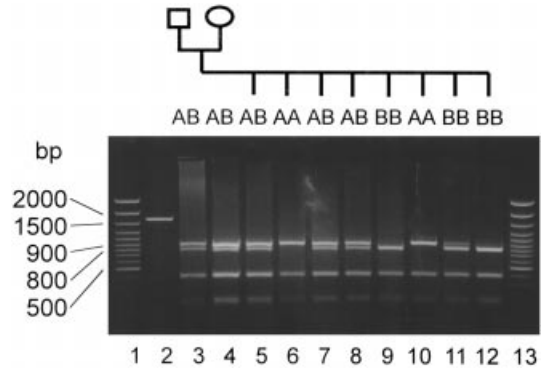

Fig. 1. Codominant segregation of *IGF2* alleles in one pig family. PCR fragments of *IGF2* digested with *Nci*I were separated on 2% agarose gel. Lanes: 1, 13, 100 bp ladder (3000, 2000, 1500, 1200, 1031, 900, 800, 700, 600, 500, 400, 300, 200, 100 bp); lane 2, PCR product; lanes 3–12, *Nci*I digested PCR products.

Chromosomal location: The porcine *IGF2* gene has been mapped to chromosome 2p1.7[1]

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Broad taxonomic applicability of microsatellites developed for the highly polymorphic neotropical cichlid, *Amphilophus citrinellum*

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Neotropical cichlids are some of the most important food fishes of Central America[1]. In addition to its important economic role as part of the freshwater fishery, the Midas cichlid, *Amphilophus citrinellum*, exhibits a high level of intraspecific variation in both coloration[1] and pharyngeal jaw morphology[2], which has made it a model species for the study of incipient, possibly, sympatric speciation[2,3]. While behavioural and ecological studies of *A. citrinellum* have been numerous and thorough, investigations of the genetic variation of this species are almost completely missing. We have previously found exceedingly low levels of variation in the mitochondrial control region and cytochrome *b* gene which suggests a recent origin of the species (Meyer *et al.* unpublished data).

While a suite of microsatellite loci have been developed for African cichlids, these microsatellite primer sets have proven largely ineffective in amplifying Neotropical species[4,5,6]. In this study, we describe the identification of six di-and tri-nucleotide microsatellite loci in *A. citrinellum* that can also be amplified in many species of cichlids both from the Neotropics and the Old World.

Genomic DNA was extracted from a single *A. citrinellum* specimen collected from Lake Nicaragua using a previously published extraction protocol[7]. *Eco*R1-digested DNA fragments were ligated to pUC18 (Gibco BRL) and transformed into SURE cells (Stratagene). The resulting library was screened with a [γ-^{32}P]-ATP end-labeled $(GT)_{10}$ oligonucleotide using standard hybridization techniques. Twenty-five positive clones of 300–1000 bp were sequenced using a *Taq* Dye-Deoxy Termination Cycle Kit (ABI – following manufacturer's recommendations) and analyzed with an ABI 373 Stretch DNA automated sequencer. Primer sets were developed for six of the 25 clones containing GT-microsatellites and adequate single-copy flanking DNA for primer design.

Amplification of the six microsatellite loci was carried out in a Geneamp 9700 Thermocycler (ABI) using 25 μl reaction volumes (Tris 67 mM, pH. 8·8; 1·5 mM $MgCl_2$; 0·4 mM of each dNTP; 75 ng of each primer and one unit of AmpliTaq DNA Polymerase (Perkin-Elmer Cetus)). Forward primers were labelled with tetrachloro-6-carboxyfluorescin (TET). Amplification reaction conditions consisted of an initial denaturing step of 3 min at 94 °C followed by 30 cycles of 94 °C for 1 min, an optimised annealing temperature (see Table 1) for 30 s, and 72 °C for 1 min. PCR products were visualised on agarose gels stained with ethidium bromide and diluted according to their strength. One microlitre of each sample was then mixed with 2 μl formamide and 0·5 μl each of size standard (GeneScan TAMRA-500, Applied Biosystems) and TAMRA buffer. The samples were denatured at 98 °C for two minutes, loaded on a 5% denaturing 19 : 1 acrylamide:bisacrylamide gel and analysed using an ABI 373 A Stretch Automated Sequencer. Allele sizes were determined by the GeneScan software (Perkin Elmer) based on comparison of migration distances with the TAMRA ladder fragments of known size within each lane.

The six microsatellite loci were amplified in a total of 140 *A citrinellum* individuals from four lacustrine populations in Nicaragua. Levels of intraspecific variation varied considerably amongst loci, with allele numbers at each locus ranging from 1 to 26 and observed heterozygosity ranging between 0·000 and 0·664.

In addition to a high overall level of intraspecific variation, these six microsatellite loci have also proven useful in a broad taxonomic array of cichlid species (Table 2). In contrast to the majority of microsatellites identified in African species that fail to amplify Neotropical cichlids[5], the present six microsatellite loci amplify both Neotropical and Old World species. These markers may prove effective in a further taxonomic clarification of relationships between New World and Old World cichlids.

The high intraspecific variation of these microsatellites makes them ideally suited to a detailed molecular investigation of observed anatomical and behavioural polymorphism in *A. citrinellum and* to molecular characterisation of wild stocks of the species. At the same time, the broad taxonomic applicability of these markers offers an opportunity to further examine evolutionary questions related to the rapid speciation of cichlid fishes.

Table 1 Primer sequences and core repeat structure for *Amphilophus cichlasoma* microsatellites. All loci were tested on 140 individuals from four lacustrine populations in Nicaragua

Locus	No. of alleles	H_O	H_E	Primer sequence (5′–3′)	Cloned repeat motif	Size of sequenced product (bp)	Annealing temperature (°C)
*Acit*1	6	0·101	0·487	**F** AAA TGA GTT CAG CGA TGG CTG AG **R** TGC ACA TCA TGT CCG CCG AAC A	$(AG)_{11}$	168–174	49
*Acit*2	26	0·593	0·926	**F** GGC ACT GAG GAT TTA TAT TAC AGG **R** GAG GTC CAG CTG AGA ACA GGG	$(GT)_{35}$	184–232	52
*Acit*3	18	0·664	0·905	**F** CTT AAG GTG TAC CTG CTT AGC **R** GAG TGG GAA GAC AGA TGT TGA GG	$(GT)_{32}$	161–195	51
*Acit*4	15	0·593	0·829	**F** CCT TCC TAC TAG TTA GTC TTT CAC **R** CAC ATA GCA CAG TGC ATT CAC CC	$(GT)_{22}$	347–375	49
*Acit*5	1	0·000	0·000	**F** GCC GCA CCC TCA TTA TCC TCA C **R** GTG ACT CCA ACG TGT AGC TTC C	$(AGC)_8$	157	52
*Acit*6	1	0·000	0·000	**F** GAA TTC ACA AAG GCC AAT CCT AC **R** GGA TAC TGA GCA TGA CAA TAA GC	$(CA)_9(TA)_{16}(TG)_{18}(TTA)_3$	268	50

Genbank accession numbers: AF237713–AF237718.
H_O = observed heterozygosity; H_E = expected heterozygosity.

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Table 2 Success of cross-species amplification of *Amphilophus citrinellum* microsatellite loci

	*Acit*1	*Acit*2	*Acit*3	*Acit*4	*Acit*5	*Acit*6
Neotropics						
Amphilophus citrinellum	+	+	+	+	+	+
Cichla cichla	+	+	+	+	+	+
Crenicichla saxatilis	+	+	+	+	?	+
East Africa						
Astatoreochromis alluaudi	+	–	+	+	+	+
West Africa						
Hemichromis bimaculatus	+	–	+	–	–	+
Madagascar and India						
Etroplus maculatus	–	+	–	+	?	–

(+) product; (–) no obvious product (?) product of questionable size.

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Two polymorphic markers for the horse *SLC11A1* (*NRAMP1*) gene

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Source/description: Primers (EqNRAMPF and EqNRAMPR), based on two conserved sites within the coding regions of the exons 5 and 6 of the human[1], bovine[2], sheep[3], and porcine[4] *NRAMP1* gene, now known as *SLC11A1,* were designed and used to amplify a product of expected size of 547 bp (PCR I). Individual samples of genomic DNA from 8 different animals of 4 different breeds (Warmblood, Old Kladruber, Arab, and Shetland Pony) were used for the PCR. The PCR products were cloned into the pCR TOPO vector (Topo TA Cloning Kit, Invitrogen, USA), and sequenced with an ABI310 Automatic Sequencer. Analysis of 11 PCR I clones obtained from 8 different animals of 4 different breeds (Warmblood, Old Kladruber, Arab, and Shetland Pony) revealed the existence of a DNA sequence homologous to exons 5 and 6 of the *SLC11 A1* gene of other species. Based on alignments with Genbank sequences reported for man, cattle, sheep and pig (Genbank accession numbers L32185, U12862, AF005380, and U55068, respectively)[1–4], the intron boundaries and the corresponding parts of exons 5 and 6 could be identified. The horse sequence showing a high level of sequence identity with other species was therefore considered part of the putative horse *SLC11 A1* gene. In all 11 clones analysed, the partial horse exon sequences were identical. The amino acid position corresponding to the nucleotide 169 substitution in intracellular parasite resistant or susceptible mouse inbred strains[5] is occupied by a glycine in the horse, identical to the resistant mouse allele and all other species examined so far. Two allelic exon 5–intron–exon 6 sequences, confirmed from at least two independent PCRs, were identified in related animals (Genbank AF163297, and AF163298). Therefore, another pair of oligonucleotides (I5ENRF and I5ENRR), amplifying the polymorphic region was designed (PCR II). The PCR II product of expected size (250 bp) was obtained.

Primer sequence:

PCR I

Primer EqNRAMPF: 5′-GCATTCTCCTCTGGCTGACC-3′

Primer EqNRAMPR: 5′-CGAGGAAGAGGAAGAAGAARGTGT-3′

PCR II

Primer I5ENRF: 5′-CAATCTGCTCTCAGCTGGACGGTAC-3′

Primer I5ENRR: 5′-GATGGCTGCGTGACTGACTT-3′

PCR conditions: Both PCRs were performed in a total volume of 12·5 μL. The PCR I mixture consisted of: 100 ng genomic DNA, 50 pmol of each primer (EqNRAMPF, EqNRAMPR), 0·2 mM of dNTPs, 1·5 mM $MgCl_2$, 0·5 U of *Taq* polymerase with 10× reaction buffer, and acetamide added to a final concentration of 5%. Reactions were run on an MJ Research DNA thermocycler (Watertown, MA) with a hot start of 2 min at 94 °C (Promega Taq Polymerase). The conditions for amplification were 30 cycles of 30 s at 94 °C, 1 min at 62 °C, and 90 s at 72 °C. The program was completed by a final extension of 30 min at 72 °C followed by cooling the reaction mixture to 4 °C. The long extension time was needed for subsequent TA cloning of the PCR product.

The PCR II mixture contained: 50 ng DNA, 20 pmol of each primer (I5ENRF and I5ENRR), 0·2 mM of dNTPs, 1·5 mM MgCl2, 0·5 U of *Taq* polymerase. The temperature cycling was carried out by an initial denaturation of 2 min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 30 s at 65 °C, then 30 s at 72 °C, with a final extension of 10 min at 72 °C and cooling to 4 °C.

Polymorphisms: The 250 bp PCR II product was digested with two restriction enzymes, *Mnl*I and *Msp*I, selected according to the nucleotide sequence polymorphisms previously identified. The gels were run at 300 V for 30 min on a 6% polyacrylamide gel (37·5 : 1), and silver stained. Two bi-allelic RFLPs were identified by analysis of sequenced clones as well as of genomic DNA. We designated the allelic variants *MnlI.1, MnlI.2, MspI.1,* and *MspI.2,* respectively. The *MnlI.1 and MnlI.2* alleles differ by absence and/or presence of a T within a polyC array, while the *MspI.1 and MspI.2* alleles differ by presence and/or absence of an A within the same array, respectively. Informative RFLP fragments for individual alleles were 48, 95 and 137 bp for *Mnl*I, and 89, 112, and 201 bp for *Msp*I, respectively. Additional small fragments were omitted. The polymorphisms detected were accompanied by a length variation of the polyC region which allowed, nevertheless, a reliable identification of the informative fragments. Homozygous and heterozygous animals were detected, and Mendelian inheritance in 2 families was confirmed (data not shown). In 27 unrelated animals of the Old Kladruber breed allele frequencies for *MnlI.1 and MnlI.2* were 0·83 and 0·17, respectively, while the frequencies of alleles *MspI.1,* and *MspI.2* in the same animals were 0·48 and 0·52, respectively. Family RFLP analysis confirmed existence of the two haplotypes (*MnlI.1-MspI.2 and MnlI.2-MspI.1*) identified by sequencing, and revealed existence of another haplotype, *MnlI.1-MspI.1.*

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A PCR test for mitochondrial heteroplasmy in sturgeon

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Source/description: Sturgeon mitochondrial DNA has extensive length variation in the D-loop. Individuals are homo- or heteroplasmic for any number of the discrete length variants of mtDNA[1]. Such length variants and/or heteroplasmic copies are useful markers for maternal lineages. Traditionally, RFLP fragments between 1·6 kb and 2·2 kb have been used to analyse heteroplasmy in sturgeons [2–4]. These long fragments resulted in different interpretations of heteroplasmy in the past[3,4]. In order to address the difficulties of analysing long RFLP-fragments we developed a PCR test using primers located in the mitochondrial $tRNA^{Pro}$ gene and in the D-loop closely related to the repeat region.

Primer sequences:

Hetero I: 5′-ACCCTTAACTCCCAAAG-3′

Hetero II: 5′-CATTTRATGGTAGATGAAAC-3′

PCR conditions: DNA was extracted from blood or tissue samples including caviar using QIAGEN kits (QIAGEN Inc., Germany). Amplifications were performed in a total reaction volume of 25 μl containing ≈ 10 ng template DNA, 2·5 μl 10 × reaction buffer, 10 pmol of each primer, 100 mM dNTPs, 2·5 mM $MgCl_2$ and 0·5 units *Taq* DNA polymerase (Oncor-Appligene, Germany) under following reaction conditions: 94 °C, 20 s; 50 °C, 10 s and 72 °C, 1 min and a final polymerisation step at 72 °C for 4 min. Electrophoresis of PCR products were run on a 1·5% agarose gel at 150 V for 2 h (Fig. 1). Lengths of repeat units were determined on a DNA sequencing automate using firm instructions (Model 310, Applied Biosystems, Foster City, CA).

Length variation: The length of the PCR products ranged between 240 bp and 673 bp depending on the number of repeats. The lengths of the individual repeat units were 78 bp in *A. mikadoi*; 79 bp in *A. oxyrinchus*; 80 bp in *A. ruthenus* and *A. sturio*; 82 bp in *A. baerii*, *A. gueldenstaedtii*, *A. persicus*, *A. naccarii*, *A. nudiventris*, *A. stellatus* and *H. huso*. Length variations of 2 bp were observed in the repeat units of *A. stellatus*, and also of 2 bp in the D-loop 5′-flanking region of *A. gueldenstaedtii*. Sequences are available in the EMBL genbank (AJ249660, AJ249662–73, AJ249675).

Heteroplasmy: The number of repeats ranged between 2 and 7, and the number of different mitochondrial genomes (copies) as distinguished by the length of the PCR products ranged between 1 (homoplasmic fish) and 2–4 (heteroplasmic fish). No heteroplasmy was observed in *A. nudiventris*, *A. oxyrinchus* and *A. sturio*. In contrast to these species, frequencies of heteroplasmic fish ranged between 8% and 83% in the other sturgeon species (Table 1).

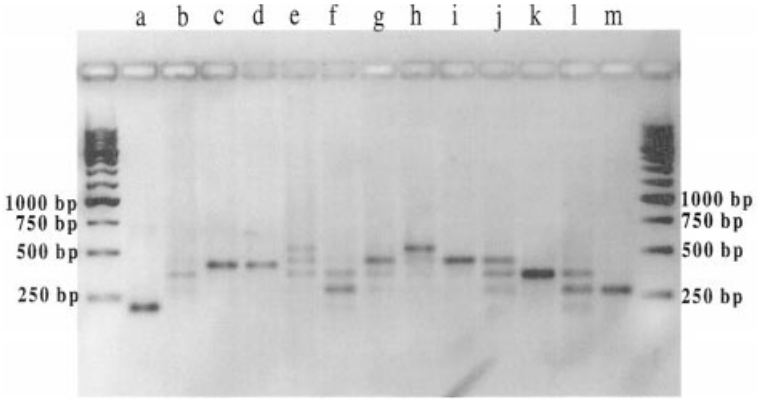

Fig. 1. Resolution of PCR products by agarose gel electrophoresis. Homoplasmic band pattern (number of repeats): a (2); c, d & i (5); k (4); m (3); heteroplasmic band pattern (number of repeats): b (3–5); e (4–6); f (2–5); g (3–5); h (5,6); j (3–5) and l (2–4). The marker is a 250-bp ladder (Boehringer, Germany).

Table 1. Species, number of sturgeons, origin of samples, and frequencies and number of homoplasmic and heteroplasmic sturgeons, frequencies and number of heteroplasmic fish in relation to the number of different copies as defined by the length variants*

			Homoplasmic		Heteroplasmic		
Species	*n*	Geographic area of sampled specimens	(*n*)	Amount (*n*)	2* (*n*)	3* (*n*)	4* (*n*)
Family Acipenseridae							
Subfamily Acipenserinae							
Genus *Acipenser*							
A. baerii	126	Siberian R., Russia	0·801 (101)	0·199 (25)	0·183 (23)	0·016 (2)	0
A. gueldenstaedtii	98	Danube R., Romania; Volga R., Russia	0·857 (84)	0·143 (14)	0·113 (11)	0·020 (2)	0·010 (1)
A. mikadoi	6	Tumnin R., Russia	0·166 (1)	0·834 (5)	0·334 (2)	0·500 (3)	0
A. naccarii	20	Buna R., Albania; Po R., Italy	0·700 (14)	0·300 (6)	0·200 (4)	0·100 (2)	0
A. nudiventris	15	Caspian Sea, Safid Rud, Iran; Rioni R., Georgia	0·666 (15)	0	0	0	0
A. oxyrinchus	60	St. John R., Canada	1·000 (60)	0	0	0	0
A. persicus	17	Caspian Sea basin	0·765 (13)	0·235 (4)	0·235 (4)	0	0
A. ruthenus	156	Volga system, Ob R. & Kuban R., Russia; Danube R., Romania, Hatchery	0·532 (83)	0·468 (73)	0·372 (58)	0·090 (14)	0·006 (1)
A. stellatus	93	Volga R. & Kuban R., Russia; Danube R., Romania, Hatchery	0·742 (69)	0·258 (24)	0·226 (21)	0·032 (3)	0
A. sturio	44	Gironde R., France	1·000 (44)	0	0	0	0
Genus *Huso*							
H. huso	74	Caspian Sea, Danube R., Hatchery	0·920 (68)	0·080 (6)	0·080 (6)	0	0

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Mapping of the oncogene c-myc (*MYC*) and the breast cancer susceptibility gene (*BRCA2*) in the pig by FISH

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Source/description: Both genes mapped here are of interest in the field of carcinogenesis. The nuclear oncogene c-myc (*MYC*) which encodes a transcription factor plays an important role in cell proliferation. Activation of the c-myc gene through mechanisms including chromosomal translocation, gene amplification, proviral insertion, or mutation may cause cancer[1]. The breast cancer susceptibility gene (*BRCA2*) appears to act as a tumour suppressor gene that participates in transcriptional regulation and DNA repair[2]. Mutations in the *BRCA2* gene are associated with an increased risk of breast cancer and other tumour types in human including ovarian, prostatic and pancreatic cancer[3]. The probes for the porcine *MYC* and *BRCA2* genes were prepared by means of PCR using primers designed on the basis of porcine gene sequences (NCBI accession numbers X97040 and Z75667, respectively). Two pairs of primers 5′-AGGGCTATACAGAGGCTTGG-3′, 5′-CGAGGTCA-TAGTTCCTGTTGG-3′ and 5′-AACGTCAGCTTCACCAACAGG-3′, 5′-TGAGGTTGCACTGGATCATGC-3′ were used for the amplification of the 2244 bp and 2563 bp long fragments which span 4773 bp of the c-myc gene. Primers 5′-CCAGGATGTTTCTCTTCAAGC-3′ and 5′-TGTAGGCTTTCTCTGTTTGGG-3′ amplified the 2613 bp long fragment of the exon 11 of the *BRCA2* gene. After their verification by restriction analysis, the PCR products were cloned in the plasmid vectors pMOS and pUC19.

Fluorescent in situ hybridisation (FISH): Both the recombinant plasmids containing two different segments of the c-myc gene were labelled with biotin-dUTP by nick-translation and used together as the hybridisation probe. The probe for the *BRCA2* gene was PCR-labelled with biotin-16-dUTP[4] and treated with DNase I to reduce the average fragment size. After *in situ* hybridisation the biotin labelled probes were detected by two layers of avidin-FITC[5]. The chromosomes were counterstained with DAPI and propidium iodide. DAPI produces G-like banding in pigs which allows the identification of chromosomes.

Chromosomal location: Precise localisations of the genes were determined on the basis of FLpter values using computer-assisted image analysis. The *MYC* gene was mapped to pig chromosome 4p14. Of the 55 metaphases analysed, 30 (55%) showed symmetrical double spots on at least one copy of chromosome 4p14. No paired signals were repeatedly detected on any other chromosomal region. The background was low. The c-myc gene had previously been assigned to human chromosome 8q24·12–q24·13 by FISH[6] and to feline chromosome F2q21·2 by FISH[7]. Therefore assignment of the c-myc gene to porcine chromosome 4p14, as reported here, is consistent with ZOO-FISH data showing conserved synteny between human chromosome 8 and this porcine chromosome[8].

The *BRCA2* gene was mapped to pig chromosome 11p13. Of the 117 metaphases analysed, 20 (17%) showed symmetrical double spots on at least one copy of chromosome 11p13. No paired signals were repeatedly detected on any other chromosomal region. The background was low. The *BRCA2* gene had previously been assigned to human chromosome 13q12–13 by linkage search[3] with the more precise localisation on 13q12·3[9]. Therefore assignment of the *BRCA2* gene to porcine chromosome 11p13, as reported here, is consistent with data showing conserved synteny and gene order on human chromosome 13 and this porcine chromosome[8,10].

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*Hpa*II PCR-RFLP within a Bov-A2 element in the promoter of the bovine *CYP21* (steroid 21-hydroxylase) gene

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Source/description: Steroid 21-hydroxylase (CYP21) is involved in the synthesis of steroid hormones and its deficiency is associated with several metabolic disorders such as human adrenal hyperplasia[1] (OMIM entry 201910). An entire Bov-A2 SINE (short interspersed nucleotide element) is located in the promoter region of the bovine CYP21 gene. PCR primers were designed for the amplification of a 342-bp fragment containing the Bov-A2 element from position 1285–1625 of the published sequence[2,3] (accession number M11267). The gene is homologous to human functional CYP21, previously designated *CYP21B*, and encodes a protein (accession number gi/163469) which shares 80·4% sequence identity to the human protein (accession number gi/2347138). Direct sequencing of the amplification products revealed several polymorphisms (Table 1), and in particular a T or C mutation at position 1564 which creates a HpaII polymorphic site. The new sequences were deposited in the GeneBank database (accession number AF163098 and AF163767).

Primer sequences:
Forward: 5′-CCCACCGAGTCCTGCCAC-3′
Reverse: 5′-GTTGAAGGACTTAAAGGAGA-3′

Table 1. Detected polymorphisms

	Sequences		
Position	M11267	AF163767	AF163098
1346	T	C	C
1348	T	G	G
1375	A	G	G
1407	T	C	C
1408–9	–	C	C
1420	T	C	C
1429	T	G	G
1456	C	A	A
1467	A	–	–
1521–2	–	C	C
1540	A	G	A
1564	T	T	C

PCR/RFLP conditions: PCR reactions were carried out in a 20-μl volume containing 10 mM Tris–HCl pH 8·3, 50 mM KCl, 1·1 mM $MgCl_2$, 0·01% gelatin, 100 μM of each dNTP, 25 ng of forward primer, 100 ng of reverse primer, 200 ng of genomic DNA and 1 unit of Taq DNA polymerase (Sigma, St. Louis, MO). We used a hot-start with the following cycling conditions: 92 °C for 10 s, at the annealing temperature for 10 s, and at 72 °C for 1 min. The annealing temperatures were 60 °C for the first 10 cycles, 55 °C for the following 20 cycles and 50 °C for the last 10 cycles. The reaction mixtures were finally incubated at 65 °C for 10 min and stored at –20 °C. Aliquots of 10 μl of the amplification reactions were digested with *Hpa*II and analysed on a 3% (w/v) agarose gel (1·5% SeaKem GTG, 1·5% Metaphor, FMC Bioproducts, Rockland, ME).

Allele frequencies: The frequencies of the *A* and *B* alleles, respectively without and with the *Hpa*II site, are shown in Table 2 for Italian Holstein Friesian, Grey Alpine, Friuli Red Pied and Reggio breeds.

Chromosomal location: The *CYP21* gene has been assigned to bovine chromosome 23 by linkage analysis[4].

Mendelian inheritance: Codominant Mendelian inheritance of the two alleles was observed in a six-member Italian Holstein Friesian family obtained by embryo-transfer.

Table 2. Allele frequencies

	Breeds (n = 20)			
Allele	IHF	GA	FRP	RE
A	0·525	0·775	0·325	0·553
B	0·475	0·225	0·675	0·447

IHF, Italian Holstein Friesian; GA, Grey Alpine; FRP, Friuli Red Pied; RE, Reggio.

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Four PCR-RFLPs and a sequence polymorphism in the porcine c-myc proto-oncogene and confirmation of the chromosomal localisation on SSC4 by linkage mapping

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Source/description: DNA samples were prepared from the breeds Pietrain (PI), Belgian Landrace (BL), German Landrace (DL), Large White (LW), Duroc (DU), Hampshire (HA), Wild Boar (WB), Mangalitza (MA) and Meishan (ME).

PCR-amplification of genomic c-myc fragments: c-myc specific PCR amplifications were performed on 250 ng genomic DNA in 50 μl reactions containing 1·5 mM $MgCl_2$, 20 mM Tris–HCl, 16 mM $(NH_4)_2SO4$, 100 μM dNTPs, 200 nM of each primer and 2·5 U *Taq*-polymerase. Primer sequences and reaction conditions are specified in Table 1.

Sequencing of PCR-fragments: PCR-products were prepared on low melting gels, isolated and directly sequenced on an automated laser fluorescent sequencer (Perkin Elmer, Weiterstadt).

PCR-RFLP detection: Sequences from ME (n = 4) and PI (n = 4) were screened for variations. Polymorphisms were verified by digestion of 10 μl of the amplification products with the appropriate endonucleases at 37 °C.

Polymorphisms: In four PCR-fragments of c-myc, five variant positions have been observed among the 198 pigs screened (Table 2).

Mendelian inheritance: Mendelian inheritance was confirmed for all polymorphisms in crosses of ME × PI.

Frequencies: In 28 pigs, the *B*-allele of the *A*-polymorphism had a frequency of 0·31. It was mainly found in the breeds Meishan (n = 6) and Mangalitza (n = 2). The *B*-allele of the C-polymorphism was detected exclusively in Meishan (n = 6). All other breeds and the Wild Boars (n = 24) had only the *A*-allele. The *B*-alleles of H_1- and H_2-polymorphisms were found exclusively in the Mangalitza breed (n = 2). The frequency of the *B*-allele in the F-polymorphism was 1·7% in DL (n = 88), 9·3% in Hampshire (n = 16), 10·0% in BL (n = 10), 12·4% in PI (n = 164), 18·05% in LW (n = 158), 22·2% in DU (n = 19), 72·5% in WB (n = 21), 91·65% in Meishan (n = 6) and 100% in Mangalitza (n = 2).

Linkage analysis was performed using CRI-MAP version 2·4 software[2], based on the *Hpa*II-RFLP of the F-fragment. The polymorphism was informative in the three F_2-families (ME × PI, WS × PI, WS × ME; total n = 943 animals), developed at the University of Hohenheim. The CHROMPIC option of CRI-MAP was used to identify potential double crossovers. Dubious animals were regenotyped. Recombination distances are shown in Table 3.

Comments: All numbering of nucleotides is listed as in GenBank accession number X97040. The five polymorphic sites were detected by comparative sequencing of the c-myc gene. PCR-RFLPs were established for four of them. The *A*-polymorphism is located closely 5′ to the P1-promoter in a tandemly organised cytosine-tetramer, a putative enhancer element for RNA-polymerase. The H_2-polymorphism is located within the poly A1 motif at position 6124–6131. The mutation which was found solely in Mangalitza leads to the shortening of the motif by one adenosine. This polymorphism might be of relevance regarding the stability of the c-myc RNA. Neither of the polymorphisms shows any effect on the amino-acid sequence of c-myc. From the results of the linkage data, c-myc can

Table 1. Primer sequences, annealing temperatures and fragment characteristics for PCR amplifications of different c-myc regions

PCR-fragment	Primer	Sequence (5′–3′)	Annealing temperature and product length	Position according to EMBL X97040	c-myc region
A	MUA	TTCAGAACTCGCTCTCCAAGTA	56 °C; 295 bp	413–708	Promoter, between
	MLA	GAACAGCTGACCCTCCACAC			P0 and P1
C	MUC	GCTGGAAGGGGAGTGGTTC	58 °C; 298 bp	3209–3507	*Intron 1*–exon 2
	MLC	GGCAGCAACTCGAATTTCTTC			–transition
F	MUF	TTGGGTGATTTCTCTTTCCTTTC	58 °C; 322 bp	5153–5484	Intron 2–*exon 3*
	MLF	CATTCTCCTCCGTGTCCGA			–transition
H	MUH	CAACCTCACAACCTTGGCTG	58 °C; 390 bp	5902–6292	Exon 3, 3′UTR
	MLH	AACACTCACAACTTAACATTTGGCT			

Table 2. Alleles at the five variant positions of four c-myc PCR-fragments

Polymorphism (position)	Alleles				Endonucleases
	A		B		
	Sequence	Fragments (bp)	Sequence	Fragments (bp)	
A (514)	CCC	294	CCCC	295	Sequence-polymorphism
C (3323)	CTAG	298	CCAG	183 + 115	*Mae*I
F (5276)	CTGG	322	CCGG	201 + 121	*Hpa*II
H_1 (6130)	CTAG	391	ATAG	228 + 163	*Mae*I
H_2 (6124)	CCGT	391	ACGT	222 + 169	*Mae*II

be excluded as a candidate gene for the SSC4 growth and fatness QTL described by Andersson *et al.* (1994)[1].

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Table 3. Recombination distances (cM) and LOD scores between *SW489/Myc* and *Myc/SW835*

Locus 1	Locus 2	Family	Recombination distance			LOD score
			Average	Male	Female	
SW489	*Myc*	WS × ME*	19·3	15·5	27·2	21·50
		ME × PI	21·7	17·7	27·1	34·04
		WS × PI	17·9	12·2	38·5	16·03
Myc	*SW835*	WS × ME	5·2	5·5	5·1	54·20
		ME × PI	4·2	3·0	5·3	77·07
		WS × PI	13·7	15·0	3·8	38·96

*ME, Meishan; PI, Pietrain.

Linkage mapping of an *Ava*I PCR-RFLP within the porcine uncoupling protein 3 (*UCP*3) gene

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Source and description: Uncoupling protein 3 (*UCP3*) is a mitochondrial transmembrane carrier that uncouples oxidative ATP phosphorylation. With the capacity to participate in thermogenesis and energy balance, *UCP3* is an important candidate gene for fatness[1]. Porcine mRNA of the *UCP3* gene was isolated from white adipose tissue and sequenced by Werner *et al.* [2] (EMBL accession number AF095744). PCR primers were designed to give an amplification product of 691 bp from the 3′ UTR of this sequence. The PCR product of expected length was observed on an agarose gel and its identity was confirmed by sequencing.

Primer sequences:

Forward: 5′ AGG ACA CGT TCG TGT GGC ACT GA 3′
Reverse: 5′ GCC CCT GGT TCC TTT GGT CTG A 3′

PCR conditions: PCR was performed in 25 μl reactions using 50 ng porcine genomic DNA, 0·2 μM each primer, 200 μM each dNTP, 2·0 mM $MgCl_2$ and 0·8 unit of *Taq* polymerase in standard PCR buffer. After an initial 95 °C denaturation step (2 min) the PCR was carried out at 95 °C (30 s), 60 °C (30 s), 72 °C (50–80 s, extension 1 s for each cycle, the last extension 5 min) for 30 cycles. Digestion of 5–10 μl of each PCR product was performed with 2 units of *Ava*I at 37 °C overnight.

Polymorphism: A biallelic polymorphism was found with *Ava*I restriction enzyme. Allele *A*, with polymorphic restriction site absent, was represented by the 691 bp uncut amplimer while allele *B*, with polymorphic restriction site present, was epitomized by fragments 428 bp and 263 bp long (Fig. 1).

Table 1. Allele frequencies of *UCP3* gene in different pig breeds.

Breed	Number of animals	Allele	
		A	B
Black Pied Pretice	18	0·08	0·92
Duroc	11	0·00	1·00
Landrace	19	0·08	0·92
Large White	18	0·00	1·00
Norwegian Landrace	11	0·50	0·50
Pietrain	14	0·43	0·57

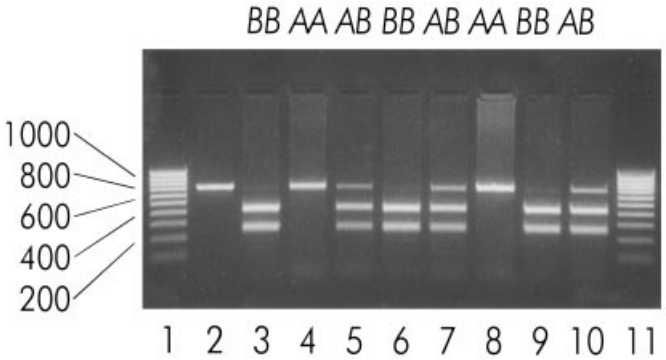

Fig. 1. Agarose gel (4%) showing different genotypes within the porcine *UCP3* gene. Lanes: 1 and 11, 100 bp ladder (1000, 900, 800, 700, 600, 500, 400, 300, 200, 100 bp); lane 2, PCR product; lanes 3–10, examples of different genotypes.

Mendelian inheritance/allele frequencies: Codominant Mendelian inheritance was confirmed in USDA-MARC backcross pedigree[3]. Allele frequencies of *UCP3* gene in unrelated animals of six pig breeds are given in Table 1.

Linkage mapping: Multipoint linkage analysis was performed in USDA-MARC backcross pedigree using CRI-MAP software package, version 2·4[4]. On the basis of 119 informative meioses the gene has been located on USDA-MARC linkage map[5] to SSC9 at position 4·5 Kosambi cM in following order: *SWR68*, *SW983*-(4·5)-*UCP3*-(4·7)-*SW21*.

Chromosomal location: The porcine *UCP3* gene was mapped by somatic cell hybrids to chromosome 9p21-p24[2,6].

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