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Genetic mapping of the ovine homologue of the mouse *Hac1* gene to sheep chromosome 1

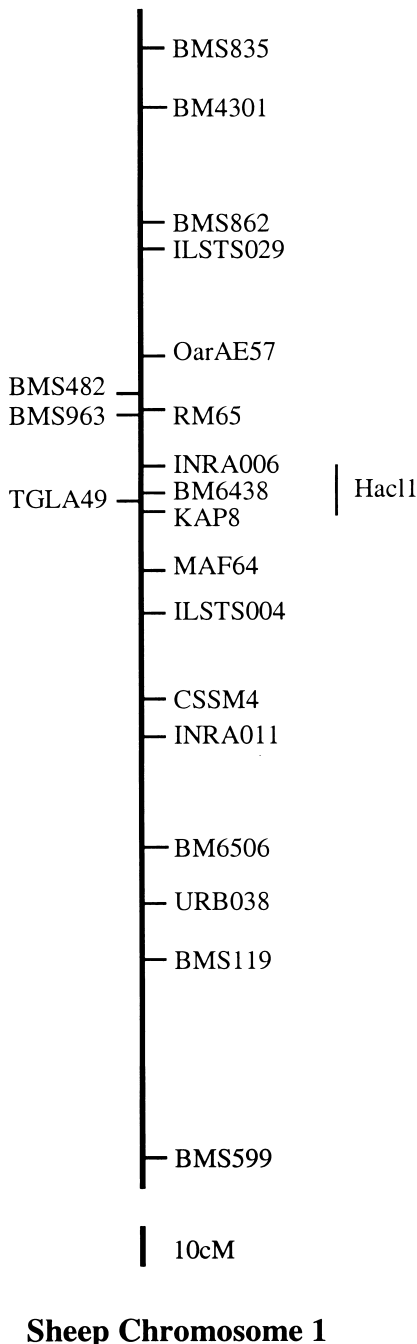


Fig. 1. Sheep chromosome 1, vertical bars indicate the most likely interval of gene loci listed on the right of the bar.

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Source/description: A cDNA clone for mouse *Hac1*¹ was used to develop an RFLP marker for use with sheep genomic DNA, in order to map the ovine homologue of this gene on the sheep genetic linkage map.

Polymorphism and allele frequency: Allelic *EcoRI* fragments of 11.5 kb (A) and 7.0 kb (B) were detected. Polymorphisms were also observed with *EcoRV* and *ScaI*. The frequency of alleles in five unrelated Merino and five unrelated Coopworth sheep was 0.85 (A) and 0.15 (B).

Method of mapping: Sheep pedigrees from the AgResearch International Mapping Flock² were used to screen for the RFLP at the *Hac1* locus and cosegregation of alleles were analysed together with other markers typed in this flock.

Hybridisation conditions: Hybridisation and washing conditions were carried out as described by Montgomery *et al.*³ using a final stringency wash at 63 °C.

Mendelian inheritance: Codominant segregation of the *Hac1* locus was verified in the AgResearch International Mapping Flock (IMF).

Chromosomal location: Multipoint linkage analysis showed significant linkage to markers on sheep chromosome 1 at LOD scores greater than 3, presented in Fig. 1 and Table 1.

Table 1. Pairwise linkage data for *Hac1*

Locus 1	Locus 2	Recombination fraction	Z_{\max}^{\dagger}
<i>Hac1</i>	INRA006	0.10	17.45
<i>Hac1</i>	BM6438	0.02	27.85
<i>Hac1</i>	TGLA49	0.00	15.05
<i>Hac1</i>	KAP8	0.03	42.09

Pairwise linkage data for hair follicle-specific gene (*Hac1*) with markers on sheep chromosome 1.

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The first microsatellite marker (*BFRO 004*) for grayling, informative for its Adriatic population A Snoj, S Sušnik, J Pohar, P Dovč

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Source/description: A polymorphic locus, characterised by dinucleotide repeat (GT)₁₁, designated as *BFRO 004* (GenBank accession number AF077764), was isolated from a library of size selected (100–600 bp) *Sau3AI* genomic restriction fragments from grayling (*Thymallus thymallus*) cloned into *BamHI* restricted pBluescript II SK+ (Stratagene) vector and propagated in Epicurian *Coli* Competent Cells (Stratagene, La Jolla, CA). Screening of the library was performed by the Chemilluminescence Quick-Light Genome Mapping Probe Kit (FMC, Bioproducts, ME) applying (CA)_n and (GA)_n oligonucleotides as probes. DNA sequencing of positive clones was performed on ABI Prism 310 Genetic Analyser using Dye Terminator

cycle sequencing kit (Perkin Elmer Applied Biosystems, Warrington, UK). Primers flanking the repeat region were designed.

Primer sequences (5'-3'):

Thy-A181 F(GT-strand): GCTCCAGTGAGGGTGACCAG (5'-fam labelled)

Thy-A181 R(AC-strand): AGCCACTGATTGAGCAGAG

PCR conditions: Amplification was carried out in 20 µl reaction volume containing 50 ng genomic DNA, 0.25 µM each primer, 0.2 mM dNTPs, 1.5 mM MgCl₂, 10 mM Tris-HCl, 50 mM KCl and 1 U *AmpliTaq* Gold DNA Polymerase (Perkin Elmer). Thermal cycling reaction was performed by initial denaturation at 94 °C for 6 min followed by 30 cycles of 1 min at 94 °C, 20 s at 55 °C, 20 s at 72 °C and 2 min final extension at 72 °C. Fluorescently labelled PCR products were run on ABI Prism 310 Genetic Analyser. Analysis and allele sizing was determined by GENESCAN Software 2.1.

Test material and polymorphism: A total of 71 blood samples of grayling were analysed. Adriatic population, reflecting a phenotypically unique variant of *T. thymallus*¹, is represented by 33 animals, the remaining 38 animals represent two geographically remote Danubian populations. Over all three alleles (157, 159, and 165 bp; Fig. 1) and five different genotypes were detected. The allele 157 was found only in grayling of the Adriatic origin, however, the other two alleles showed up in all the tested populations. The observed frequencies of these three alleles are listed in Table 1.

Other comments: There were no statistically significant differences

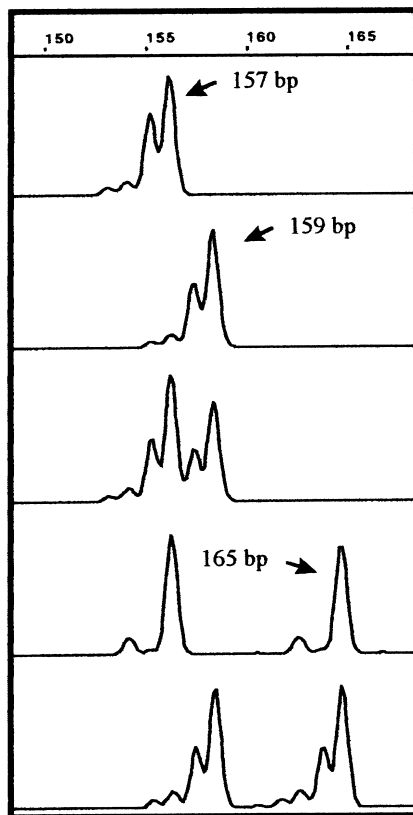


Fig. 1. Three alleles representing five different genotypes.

Table 1. Frequencies of BFRO 004 alleles ($\chi^2 = 24.38$; $P < 0.001$), found in the Adriatic and Danubian populations of *T. thymallus*

Origin	Allele			
	<i>n</i>	157	159	165
Adriatic	66	0.273	0.667	0.06
Danubian	76	0	0.868	0.132

found in allele frequencies between the two Danubian sampling locations.

Acknowledgements: We thank Dušan Jesenšek, the manager of the Tolmin hatchery, Dušan Ulčar, from the Fishery Association of Radovljica, Špela Budihna and Simon Pleško, both from the Fisheries Institut of Ljubljana, for providing samples.

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A dog microsatellite at the VIAS-D21 locus with demonstrated linkage to the marker CXX20

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Source and description: A canine genomic DNA library was constructed and screened as described previously¹. A positive clone was sequenced and identified as having a repeat of the type (GT)₃C(GT)₁₄ (GenBank AF072728). This microsatellite was named VIAS-D21 and primers were designed to allow PCR amplification. This locus was amplified and found to be polymorphic in a random population of greyhounds.

Primer sequences:

Forward: TAATGATTCTCTTTCCCGTTTCC

Reverse: CCCTCCACTCTTCCCATTGTGCTC

Mendelian inheritance: Segregation was observed in 10 greyhound and one Border Collie families. Alleles were named according to their size in base pairs by comparison to M13 sequence ladder.

Frequency: A total of 38 unrelated greyhounds were analysed to determine allele frequencies. The PIC and power of exclusion (PE) of this marker were 0.486 and 0.288, respectively (Table 1).

Table 1. Allele frequencies

Allele (bp)	Frequency
181	0.58
183	0.12
187	0.3

PCR conditions: The forward primer was end-labelled with [³²P]dATP. The PCR mix comprised of 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 0.001% gelatin (w/v) 1 U *AmpliTaq* Gold, primers at 0.2 mM and 200 mM of each dNTP. Approximately 100 ng of DNA was amplified in a 5-µl volume using the program 95 °C 10 min (95 °C 45 s, 50 °C 45 s) × 30 cycles, 72 °C 10 min using an Omnigene thermocycler (Hybaid, UK). After amplification PCR products were subjected to electrophoresis on a 6% denaturing polyacrylamide gel and visualised by exposing the gel to radiographic film.

Linkage analysis: Linkage analysis was performed against 12 other canine markers including CXX20². Linkage was analysed using eight full- or half-sib families with the number of siblings ranging from 10 to 41 per family. Analysis was performed using the ANIMAP programs (courtesy of D. Nielsen and M. Georges). The results of this analysis showed that there was tight linkage between the markers VIAS-D21 and CXX20 (maximum LOD score of 12.234 with zero recombination, $P < 0.001$).

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