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Short communication

# Polymorphisms detected in the *tyrosinase* and *matp* (*slc45a2*) genes did not explain coat colour dilution in a sample of Alpaca (*Vicugna pacos*)

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ABSTRACT

The molecular basis of the inheritance of alpaca fibre colour is poorly understood. It is likely that colour dilution is having an influence on alpaca fibre colour, with all primarily pheomelanic animals differing mainly in their total melanin concentration, as opposed to relative concentrations of pheomelanin and eumelanin. Candidate genes to cause such dilution include *tyrosinase* (*tyr*) and *membrane associated transport protein* (*matp*), both of which have been associated with coat colour dilution in other species. PCR primers were designed in regions surrounding each exon of *tyr* and *matp* and these exons were sequenced in 24 animals with dilute or non-dilute colour phenotypes from around Australia. No polymorphism found in the coding region of any *tyr* or *matp* exon was associated with dilution in fibre colour.



## 1. Introduction

Outside of South America alpacas are bred mainly for their fibre, which is highly prized in the global textile market (Frank et al., 2006; McGregor, 2006). Alpaca fibre is highly valued because it is strong and suitable for use in high quality clothes, fabrics and carpets (Frank et al., 2006; Lupton et al., 2006; McGregor, 2006). White alpaca fibre is considered the most valuable because it is easy to dye (Frank et al., 2006).

The melanin synthesis pathway has been demonstrated to be highly conserved in mammals (Deng et al., 2007; Wang and Hebert, 2006; Guibert et al., 2004; Schmutz et al., 2004; Camacho-Hübner et al., 2002; Martinez-Arias et al., 2000). This knowledge assists in the nomination of possible mechanisms that could be pivotal to alpaca pigmentation. A number of different enzymes and ligands are involved in the melanin synthesis pathway and will have some bearing on colour (Sturm et al., 2001; Busca and Ballotti, 2000). These include both the Agouti Signalling Protein (ASP) and the Melanocortin-1 Receptor (MC1R), which are involved in switching between eumelanin and pheomelanin pigment production (Oyehaug et al., 2002). Other enzymes include tyrosinase (TYR) and related proteins, as well as the membrane associated transport protein (MATP); mutations in which have been associated with dilutions in eumelanin and pheomelanin concentration, and thus with fibre colour intensity; for example both genes are associated with albinism in humans (Newton et al., 2001; Oetting et al., 1996).

Both *tyr* and *matp* have known links to colour dilutions in a number of species. *Tyr* mutations that result in albinism have been demonstrated in rabbits (Aigner et al., 2000), cattle (Schmutz et al., 2004), cats (Imes et al., 2006), rats (Blaszczyk et al., 2005), ferrets (Blaszczyk et al., 2007) and minks (Anistoroaei et al., 2008). Along with the albino phenotype, many other *tyr* alleles have been identified to cause white or near-white phenotypes, the most noted of which are the chinchilla, himalayan, chinchilla mottled and platinum phenotypes in mice (Beermann et al., 2004; Jackson et al., 1994). Mutations in the *matp* gene have also been shown to result in colour dilution, notably the underwhite dilution in mice (Bennett and Lamoreux, 2003; Newton

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et al., 2001), buckskin and palomino in horses (Mariat et al., 2003), ocutaneous albinism type 4 in humans (Newton et al., 2001) and other minor variations in human pigment (Graf et al., 2005).

Previous research has demonstrated that alpacas with the same genotype at the *mc1r* gene can exhibit different phenotypes, for example ranging from light to dark fawn (Feeley and Munyard, 2009). This being the case, colour dilution genes such as *tyr* and *matp* provide a logical alternative avenue to explore in relation to alpaca colour genetics.

## 2. Materials and methods

Blood (approximately 5 mL) was taken from 24 alpacas of both sexes, with a variety of different colour phenotypes, from various breeders across Western Australia, New South Wales and Victoria. The phenotypes selected placed an emphasis on potentially diluted phenotypes such as pink skin (PS) white and PS light fawn and also included non-fading and fading black, black and tan, bay, chestnut, black skin (BS) brown, PS rosegrey, BS silvergrey, BS white and BS blue-eyed white. These phenotypes were then sorted into dilute and non-dilute colour types, with 15 animals showing dilute phenotypes and nine animals the non-dilute phenotype.

DNA was extracted from alpaca blood using the salt precipitation method described by Miller et al. (1988). Where this method failed to yield sufficient quantities of DNA, or where the quantity of blood obtained was insufficient for the salt precipitation method, the DNeasy blood and tissue DNA extraction kit (Qiagen) was used, according to the manufacturer's instructions.

Where possible alpaca *tyr* and *matp* sequences were retrieved from sequences deposited in GenBank (NCBI), using BLAST analysis against known cattle exon sequences. Primers were then designed in regions flanking each exon (Table 1). Where the trace archive sequence was incomplete (for *matp* exons 1, 2 and 6) a comparative genetics approach was adopted. Sequences from cattle, dogs, humans and mice were aligned, and primers were designed using sequence from consensus regions flanking each exon.

Genomic DNA (50–100 ng) was used as template for PCR using 0.2 μM of forward primer, 0.2 μM reverse primer, 1× polymerisation buffer (Fisher biotec), 2 mM $MgCl_2$ (Fisher biotec) and 0.75 um BIOTAQ polymerase (Bioline) in a 10 μL reaction. Optimized amplification conditions were: 95 °C for 2 min; 30–40 cycles of 95 °C for 20 s, 57–58 °C for 30 s and 72 °C for 1 min; then 72 °C for 5 min. *Tyr* exon 1 and *matp* exons 2–5 were amplified using 30 cycles with 58 °C annealing temperature; *tyr* exons 2, 4 and 5 and *matp* exon 6 were amplified using 30 cycles with 57 °C annealing temperature; *tyr* exon 3 was amplified using 40 cycles at 58 °C annealing temperature, *matp* exon 1 was amplified using 35 cycles at 57 °C annealing temperature; and *matp* exon 7 was amplified using 35 cycles at 58 °C annealing temperature.

PCR products were amplified from genomic DNA in five separate 10 μL reactions and pooled prior to sequencing. Given the length of the *tyr* exon 1 amplicon, internal primers were needed to obtain the full sequence (TYR1iL: 5′-CATCTTTGATTTGAGTGTCC-3′ and TYR1iR: 5′-ATGTCACTGAACATGGGTG-3′). Sequencing was performed using the ABI Big Dye Terminator® system at either Murdoch University, Perth, using a 48-capillary ABI 3730 DNA analyser, or at Macrogen, Korea, using 96-capillary ABI 3730XL DNA analysers. Vector NTI software (Invitrogen) was used to visualise and analyse sequencing results and SpliceView (ORIEL) was used to aid in determining intron/exon boundaries for predicted proteins. Statistical analyses were performed using the JMP 7 statistical package (SAS Institute Inc., 2007). A tree regression tree model, with a categorical response variable (SNP), was fitted to the data. The Pearson $\chi^2$ test was then used to investigate if an association existed between fibre colour and any of the SNP's found. Statistical significance was accepted at $\alpha = 0.05$.

**Table 1**
PCR primers used to amplify *Tyr* and Matp exons.

| Primer name | Sequence (5′–3′) | Amplicon length (bp) |
|---|---|---|
| Tyr1L | GAGAGTGTTTGATGCTGGAG | 1118 |
| Tyr1R | GGAAAGATGAGAAAGGAGGT | |
| Tyr2L | TGAATGAAGAAAGAGATTACC | 417 |
| Tyr2R | AGTGGTAGATGCAAGACTCG | |
| Tyr3L | TTTACATTGCCCTAACGACT | 385 |
| Tyr3R | GAATCCCAAGACAGAATGAA | |
| Tyr4L | TTTAATTCCAGTGCTCTTCC | 483 |
| Tyr4R | ACTGTATTTGGCATCTGTCC | |
| Tyr5L | CTCCAAAGTGCGATGTTAGT | 463 |
| Tyr5R | TTCTACCTGAGTTGGAGGAA | |
| MATP1L | TAACTTGTGATCTTAGAGGC | 700 |
| MATP1R | GTCGTTTCTAAAAGTAAAGG | |
| MATP2L | GGAGATAAGTTAATGAGAGG | 418 |
| MATP2R | AGACAGAGCCCGCGTGATGG | |
| MATP3L | GCTTCTCCAGTCATAAGCAG | 552 |
| MATP3R | GCTCCTGGAGATTTTAGGAT | |
| MATP4L | TGTGTAATATGGCTGTGTGC | 329 |
| MATP4R | CTGGAACCTGTCTGTAAGC | |
| MATP5L | AGGTGTGAAAGGATGAAATG | 371 |
| MATP5R | GAATAGCTTTTCCTAATCTCCA | |
| MATP6L | GTTGTGAGGCACYGCYAGCT | 344 |
| MATP6R | CTGCTCTACACCTTCCTACC | |
| MATP7L | ATTGATTTCTTGCTGCGTGC | 262 |
| MATP7R | TTATTATCTGGCTTTGTACAG | |

## 3. Results

The five exons of the *tyr* gene and the seven *matp* exons were sequenced in 24 different animals, separated into two groups. While *tyr* exon 2 was largely sequenced only in one direction due to difficulties sequencing through an adjacent microsatellite, all other exons were sequenced in both directions for the majority of alpacas. Ten single nucleotide polymorphisms (SNPs) were detected in the coding region of *tyr*. Seven of the SNPs were synonymous polymorphisms and three were non-synonymous (Table 2). Two SNPs were detected in the 7 exons of the *matp* gene. One of these was synonymous and the other was non-synonymous (Table 3).

There was no correlation found when comparing the two phenotypes (dilute and non-dilute) with any individual SNP from either gene. When phenotype was compared to multiple SNP's however correlations did appear. The