Coat colour genetics: application to Maremmano horse breeding program

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Objective

Our aim is to determine the coat colour genetics in Maremmano horse population to help selective breeding for black and against chestnut as requested by ANAM (Maremmano horse breeders' association).

Introduction

The Maremmano horse has a Stud Book since 1980. This breed is used for Saddle horse production, as purebred or crossbred (Italian Saddlebred). The admitted coat colours are: black, bay and, only for female, chestnut. The extension (*E*) and agouti (*A*) loci determine the relative amount of red/yellow pigment (phaeomelanin) and black pigment (eumelanin) in mammals.

The extension locus encodes the melanocyte-stimulating hormone receptor and animals homozygous for recessive alleles at the extension locus do not express a functional MSH receptor (*MC1R*); consequently, they show a red/yellow pigmentation. In contrast, animals carrying dominant allele at the extension locus express an overactive receptor which will cause a black/bay pigmentation.

Agouti-signalling-protein (*ASIP*), encoded by Agouti locus, controls the relative amounts of melanin pigments in mammals. *ASIP*, in the presence of a functional *MC1R* (*E/E, E/e*), produces animal black (*a/a*) or bay (*A/A, A/a*).

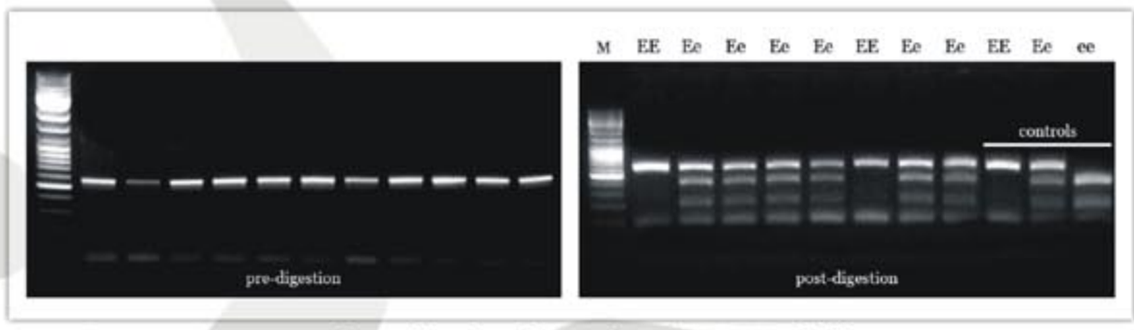
In horse, a mutation at locus *E* leads to the chestnut coat colour; this is a missense substitution at codon 83 (**TCC** to **TTC**), causing a non-conservative amino acid substitution (Ser to Phe) in the *MC1R* product associated with the *e* allele (Marklund *et al.*, 1996);

	+AA	*+Aa*	*+aa*
EE	bay	bay	black
Ee	bay	bay	black
ee	chestnut	chestnut	chestnut

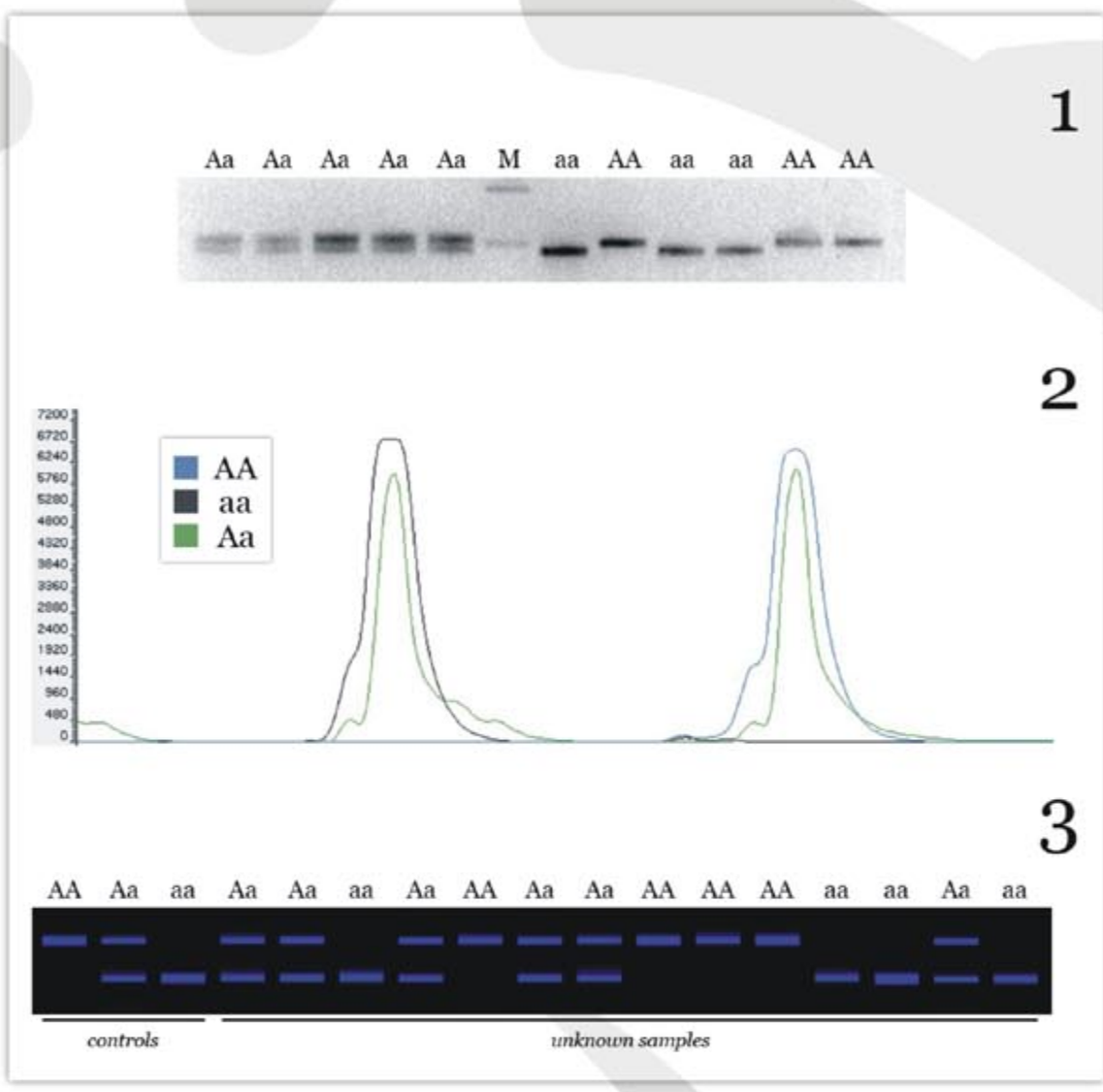
Concerning the Agouti locus, a 11 bp deletion in *ASIP* exon 2, alters the aminoacid sequence and the frameshift initiated by the deletion results in a modified agouti-signalling-protein associated with horse recessive black coat colour (Rieger *et al.*, 2001).

Methods

A total of 162 Maremmano horses, 30 stallions and 132 mares, were included in this investigation; blood samples were taken from the jugular vein with EDTA-containing vacutainers and DNA was isolated from 500 µl of whole blood using GeneElute blood genomic DNA Kit (Sigma) following the manufacturer's instructions. The *E* locus assay is based on the amplification of a 560 bp sequence that if mutated (T instead of C in position 901, *MC1R* gene X98012) creates a restriction site for *Taq*I endonuclease (NEB). Target is amplified using MC1R FOR `5'-CTACCTCGGGCTGACCACCAAC-3'` and MC1R REV `5'-GCACGTACAGCACTGCCATGAG-3'` performing 35 cycles (95°C for 45", 64°C for 45" and 72°C for 1') with 30 ng of DNA template. Digestion was performed using 20µl of PCR reaction following the manufacturer's specifications. Digested fragments are separated by electrophoresis in a 1,5% agarose gel. The *A* locus screening is based on the detection of a 11 base pair deletion in agouti exon 2 (AF288358) (Rieger *et al.*, 2001). Target is amplified using TestADEx2-F `5'-CTTTTGTCTCTCTTTGAAGCATTG-3'` and TestADEx2-R `5'-GTCAGGGTCCT-CCCCTTTTA-3'` (with the following reaction condition: 95°C for 45", 58°C for 45" and 72°C for 30", 35 times. The result is revealed by electrophoresis on a high resolution (4%) agarose gel. TestADEx2-F was also used with another reverse primer (ASIP Black REV FAM `5'-GTCAGGGTCCTCCCCTTTTA-3'`) obtaining labelled product(s) revealed with ABI PRISM 377 following the same PCR conditions.



Locus *E* agarose assay: pre and post digestion electrophoresis. M: DNA ladder.



1: Agarose electrophoresis of locus *A* assay; M: DNA ladder. **2**: GeneScan analysis of the three allele combination (see legend in the figure). **3**: Image rendering of a GeneScan results with Genographer software.

Results and Discussion

The *E* locus assay is designed to reveal, in case of homozygous mutation, two bands (184 bp and 376 bp), resulting from the digestion of the 560 bp amplicon while eterozygous individuals have a three bands pattern (184, 376 and 560 bp). In a population of 162 Maremmano horses 88 (54,4%) horses were *E/E* (not chestnut), 72 (44,4%) were *E/e* (not chestnut) and only 2 (1,2%) were *e/e* (chestnut).

For the Agouti locus, it has been evidenced the deletion of 11 bp using the two different reverse primers (see methods). The fragments resulted to be 102 bp and 91 bp (TestADEx2-F - TestADEx2-R) and 143 bp and 132 bp (TestADEx2-F - ASIP Black REV FAM) for the allele *A* and *a*, respectively.

Within the screened population 86 individuals (53%) were *A/a*, 44 (27%) were *A/A*, and 32 (20%) were *a/a*. As expected, 32 animals (*E/E* or *E/e*) homozygous for recessive alleles (*a/a*) were phenotipically black, which represent the preferred coat colour for some "traditional" breeders. However, molecular screening differentiated some dark bay erroneously considered black (data not shown). Moreover, with this molecular informations, it is possible to plan mating in order to avoid chestnut colour which is not admitted by the Stud Book for Maremmano stallion.

This investigation, sponsored by ARSIA (Agency for Development and Innovation of Agriculture Tuscany), will involve the whole Maremmano breeding stock DNA bank (about 2000 individuals). The results will be available to the breeders in the ANAM website.

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