



## Measuring and modeling for the assessment of the genetic background behind cognitive processes in donkeys



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### ARTICLE INFO

#### Keywords:

Linear model  
Ass  
Animal cognition  
Animal intelligence  
Coping styles

### ABSTRACT

New productive niches can offer new commercial perspectives linked to donkeys' products and human therapeutic or leisure applications. However, no assessment for selection criteria has been carried out yet. First, we assessed the animal inherent features and environmental factors that may potentially influence several cognitive processes in donkeys. Then, we aimed at describing a practical methodology to quantify such cognitive processes, seeking their inclusion in breeding and conservation programmes, through a multifactorial linear model. Sixteen cognitive process-related traits were scored on a problem-solving test in a sample of 300 Andalusian donkeys for three consecutive years from 2013 to 2015. The linear model assessed the influence and interactions of four environmental factors, sex as an animal-inherent factor, age as a covariable, and the interactions between these factors. Analyses of variance were performed with GLM procedure of SPSS Statistics for Windows, Version 24.0 software to assess the relative importance of each factor. All traits were significantly ( $P < 0.05$ ) affected by all factors in the model except for sex that was not significant for some of the cognitive processes, and stimulus which was not significant ( $P < 0.05$ ) for all of them except for the coping style related ones. The interaction between all factors within the model was non-significant ( $P < 0.05$ ) for almost all cognitive processes. The development of complex multifactorial models to study cognitive processes may counteract the inherent variability in behavior genetics and the estimation and prediction of related breeding parameters, key for the implementation of successful conservation programmes in apparently functionally misplaced endangered breeds.

### 1. Introduction

Being domesticated prior to the horse, the suitability of the donkey species for mankind has been documented through History. Considering its overall docile nature, donkeys have been proved to be especially suitable for women and children, who use them for traction and draught power when compared to oxen or larger equines. In areas where donkeys are no longer used, owners and breeders are left to find alternative uses otherwise endangered breeds vanish. This sets an optimal framework for new donkey application niches to arise, as for example, their use in leisure and equine assisted therapy (Rose et al., 2011), which are supported by scientifically reported beneficial effects on human health (Borioni et al., 2012). Donkeys used in such settings

must be tested and selected for their abilities to develop cognitive processes, especially those relating to their overall behavior and coping style levels, as this may translate in reducing the money and time invested in their education.

The knowledge on the factors conditioning cognitive processes is especially relevant to assess the genetic variability behind them, as it may help develop accurate selection programmes, aiming at preserving such variability, one of the keys for survival in endangered breeds.

Contrary to what authors such as Hausberger et al. (2004) have recommended, functional traits have never comprised the selection criteria included in the breeding programmes of donkeys, as only morphological and phenoptical (mainly coat) features had been considered.

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<http://dx.doi.org/10.1016/j.rvsc.2017.09.013>

Received 27 May 2017; Received in revised form 31 July 2017; Accepted 13 September 2017

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There are many internal and external factors that may affect equid behavior and therefore, the cognitive processes that equids develop. Researchers have measured how factors such as environment (French, 1993), handling conditions (Lansade et al., 2004), age, sex, breed, sire (Hausberger et al., 2004), season, diurnal cycles (Lamoot and Hoffmann, 2004) and year (Lamoot et al., 2005) may modulate donkey behavior from a phenotypical perspective. Although such factors have been reported to be significant for the development of different ethological patterns, no study has focused on assessing reliable quantitative methods for their integration in linear genetic models in donkeys. Hence, this study constitutes the first of its kind aiming at understanding the degree at which non-genetic factors influence cognitive processes under field conditions in donkeys.

The two main objectives of this study were, first, to assess the effects that inherent factors (sex and age) and external environmental factors (assessment year, season, stimuli and husbandry system) have on cognitive processes in donkeys, and second, to describe the potential implementation of quantifiable genetic models for the inclusion of such cognitive processes in breeding and conservation programmes through a routine *in-situ* test methodology.

## 2. Material and methods

### 2.1. Animals

Records from 300 Andalusian donkeys (n = 300, 78 jacks and 222 jennies), with ages ranging from 9 days to 23 years, were used in this study. All the donkeys were registered in the Andalusian donkey stud-book and had been genotyped by the use of a filiation test for each mating with 24 microsatellite molecular markers recommended by the International Society of Animal Genetics (ISAG), especially suitable for donkeys (Table 1). The donkeys (n = 300) were the progeny of 93 jacks and 253 jennies.

### 2.2. Cluster definition context: etymological reasons and scale definitional issues

Intelligence or IQ-related cognitive processes have been suggested to be influenced by environmental factors, as opposed to other cognitive processes which may not necessarily be affected. This context suggests a potential hereditary or genetic background conditioning them and lays the basis for their quantification and qualification. The strategies used to measure cognitive processes and the etymological controversy raised when we intend to sort them into categories, to isolate intelligence or coping style related ones from the rest, often arrives at a point at which, although we cannot consider these processes to be synonyms, they may often overlap.

The practical study of complex traits, such as cognitive processes, always requires the thorough separate definition of the traits being considered, as concepts may outline traits better than terms themselves. In this study, we initially separated the cognitive processes assessed into three clusters to define and study them more accurately. The first of them or coping style cluster involved three traits describing the reactivity of the donkeys to visual and auditory stimuli presented from different positions. The two remaining clusters were divided considering the differences set by Sparrow and Davis (2000). According to these authors, a second cluster or cognition cluster comprised the traits that referred to the cognitive processes whereby individuals acquire knowledge from the environment. The third cluster or intelligence cluster considered intelligence in a very narrow sense, referring to those cognitive processes that are commonly evaluated by intelligence human IQ tests or by extension, g-factor animal related tests (Boring, 1929). Sparrow and Davis (2000) would address the agreement on the existence of multiple components that combine to produce complex cognitive processes (such as problem-solving), as the common point at which the different definitions and theories of cognition and

**Table 1**  
24 specific microsatellite primers (nuclear DNA) used for genotyping and parentage tests in donkeys.

Locus	Primers (5' → 3')	Sequence length/Range (bp)
AHT4	F: AACCCCTGAGCAAGGAAGT R: GCTCCAGAGAGTTTACCCT	128–160
AHT05	F: ACGGACACATCCCTGCCTGC R: GCAGGCTAAGGAGGCTCAGC	124–154
ASB2	F: *CACTAAGTGTCTTTCAGAAGG R: CACAACAGATTCTCTGATAGG	222–256
ASB23	F: GCAAGGATGAAGAGGGCAGC R: CTGGTGGGTTAGATGAGAAGTC	134–148
UCDEQ (CA) 425	F: AGCTGCCTCGTTAATTCA R: CTCATGTCGGCTTGTCTC	222–242
HMS2	F: CTTCAGTCGAATGTGATTTAAATG R: ACGGTGGCAACTGCCAAGGAAG	225–245
HMS3	F: CCAACTCTTTGTACATAACAAGA R: CCATCCTCACITTTTTCACITTTGTT	152–170
HMS5	F: TAGTGTATCCGTACAGATTCAAAG R: GCAAGGAAGTCAGACTCCTGGA	97–111
HMS6	F: GAAGCTGCCAGTATTCAACCATTG R: CTCCATCTTGTGAAGTGAACCTCA	149–167
HSM7	F: CAGGAACTCATGTTGATACCATC R: TGTGTTGAAAACATACCTTGACTGT	167–177
HTG6	F: CCTGCTGGAGGCTGTGATAAGAT R: GTTCACTGAATGTCAAATTTCTGCT	78–84
HTG10	F: CAATTCCCGCCCAACCCCGGCA R: TTTTATTCTGATCTGTACATTT	83–103
HTG15	F: TCCTGATGGCAGAGCCAGGATTTG R: AATGTCACCATGCCGCACATGACT	116–134
LEX3	F: ACATCTAACCCAGTGCTGAGACT R: AAGAACTAGAACCTACAACTAGG	194–220
VHL20	F: CAAGTCTCTTACTTGAAGACTAG R: AACTCAGGAGAGAATCTTCTCAG	75–105
TKY287	F: ATCAGAGAACCAAGAAGG R: TCTCTGCTATAGGTAAGGTC	215–245
TKY294	F: GATCTATGTCTAGCAAACAC R: CTAGTGTTCAGATAGCCTC	210–235
TKY297	F: GTCTTTTGTGCTCGGTG R: TCAGGGGACAGTGGCAGCAG	215–250
TKY301	F: AATGGTGGCTAATCAATGGG R: GTGTATGATGCCCTCATCTC	140–170
TKY312	F: AACCTGGGTTTCTGTTGTTG R: GATCCTCTTTTTATGGCTG	90–130
TKY321	F: TTGTTGGGTTTAGGTATGAAGG R: GTGTCAATGTGACTTCAAGAAC	175–210
TKY341	F: TATCCAGTCACCCATTTTAC R: TTGTGTCAGTACACTCTATG	135–160
TKY343	F: TAGTCCCTATTTCTCTGAG R: AAACCCACAGATACCTTAGA	135–170
TKY344	F: GTGTCCATCAATGGATGAAG R: CTTAAGGCTAAATAATATCC	75–115

F: Forward primer; R: Reverse primer.

intelligence converge. This dissertation sets the main behavioral context of our study, and is one of the main reasons for the design and use of the present problem-solving test (Table 2), as it enables the simultaneous quantification and classification of the ability of the donkeys under study to develop such complexly intertwined cognitive processes.

Not only is the difficulty in isolating cognitive processes for their study, but also the fact that they may be measured differently, what determined the use of the test elected as well. IQ related or g factor (see Anderson, 2000) intelligence tests provide numerical values assigned on a scale. By contrast, although cognitive assessment does not necessarily use a numerical score, it enables categorical values to be translated into linear numerical scales, therefore connecting the quantification and qualification of the processes studied. The translations from the cognitive processes categorical scales to numerical scales for the three clusters described above are shown in Tables 3 and 4.

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