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

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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 phaneroptical (mainly coat) features had been considered.

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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 studbook 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 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.

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

Locus	Primers (5′ → 3′)	Sequence length/ Range (bp)
AHT4	F: AACCGCCTGAGCAAGGAAGT R: GCTCCCAGAGAGTTTACCCT	128–160
AHT05	F: ACGGACACATCCCTGCCTGC R: GCAGGCTAAGGAGGCTCAGC	124–154
ASB2	F:*CACTAAGTGTCGTTTCAGAAGG R: CACAACTGAGTTCTCTGATAGG	222–256
ASB23	F: GCAAGGATGAAGAGGGCAGC R: CTGGTGGGTTAGATGAGAAGTC	134–148
UCDEQ (CA) 425	F: AGCTGCCTCGTTAATTCA R: CTCATGTCCGCTTGTCTC	222–242
HMS2	F: CTTGCAGTCGAATGTGTATTAAATG R: ACGGTGGCAACTGCCAAGGAAG	225–245
HMS3	F: CCAACTCTTTGTCACATAACAAGA R: CCATCCTCACTTTTTCACTTTGTT	152–170
HMS5	F: TAGTGTATCCGTCAGAGTTCAAAG R: GCAAGGAAGTCAGACTCCTGGA	97–111
HMS6	F: GAAGCTGCCAGTATTCAACCATTG R: CTCCATCTTGTGAAGTGTAACTCA	149–167
HSM7	F: CAGGAAACTCATGTTGATACCATC R: TGTTGTTGAAACATACCTTGACTGT	167–177
HTG6	F: CCTGCTTGGAGGCTGTGATAAGAT R: GTTCACTGAATGTCAAATTCTGCT	78–84
HTG10	F: CAATTCCCGCCCCACCCCCGGCA R: TTTTTATTCTGATCTGTCACATTT	83–103
HTG15	F: TCCTGATGGCAGAGCCAGGATTTG R: AATGTCACCATGCGGCACATGACT	116–134
LEX3	F:ACATCTAACCAGTGCTGAGACT R:AAGAACTAGAACCTACAACTAGG	194–220
VHL20	F: CAAGTCCTCTTACTTGAAGACTAG R: AACTCAGGGAGAATCTTCCTCAG	75–105
TKY287	F:ATCAGAGAACACCAAGAAGG R:TCTCTGCTATAGGTAAGGTC	215–245
TKY294	F:GATCTATGTGCTAGCAAACAC R:CTAGTGTTTCAGATAGCCTC	210–235
TKY297	F:GTCTTTTTGTGCCTCGGTG R:TCAGGGGACAGTGGCAGCAG	215–250
TKY301	F:AATGGTGGCTAATCAATGGG R:GTGTATGATGCCCCTCATCTC	140–170
TKY312	F:AACCTGGGTTTCTGTTGTTG R:GATCCTTCTTTTTATGGCTG	90–130
TKY321	F:TTGTTGGGTTTAGGTATGAAGG R:GTGTCAATGTGACTTCAAGAAC	175–210
TKY341	F:TATCCAGTCACCCATTTTAC R:TTGTGTCAGTACACTCTATG	135–160
TKY343	F:TAGTCCCTATTTCTCCTGAG R:AAACCCACAGATACTCTAGA	135–170
TKY344	F:GTGTCCATCAATGGATGAAG R:CTTAAGGCTAAATAATATCCC	75–115

F: Forward primer; R: Reverse primer.

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.

Table 2
Problem-solving test phase I and II description and treatment classification.

Treatment	Description	Stimulus type	Reinforcement
Phase I. Oilcloth test.			
Treatment 1 (S1)	Handler (B) uses a lead rope and soft voice, trying to comfort the donkey to make the donkey cross the oilcloth on the floor, but without pulling the rope if the donkey refuses to move.	Unknown frontal visual stimulus.	Positive
Treatment 2 (S2)	Handler (B) used a lead rope with applied pressure to make the donkey cross over the oilcloth. Handler (B) releases the pressure when the donkey moves as it crosses the oilcloth.	Known frontal visual stimulus.	Positive
Treatment 3 (S3)	The donkey was lured by a familiar treat (dry bread, carrots or feed, depending on the owner's tastes and to which the donkey was accustomed) by handler (C). The treat is given to the donkey when the task was completed.	Known frontal visual stimulus.	Positive
Treatment 4 (S4)	Handler (B) applied pressure to the lead rope and handler C made noise from behind the donkey with a so-called “donkey motivator” (plastic bag tied on the end of a stick) (McLean et al., 2012). Handler (B) led the donkey by slightly pulling the rope until the donkey crosses the oilcloth completely.	A known frontal visual stimulus and an unknown rear auditory stimulus.	Negative
Treatment 5 (S5)	Two handlers (B and C) using two lead ropes attached on either side of the halter to encourage the donkey across. The handlers (B and C) released the pressure when the donkey moves and then reapplied when it stops until it crosses the oilcloth completely.	Known frontal visual stimulus.	Negative
Treatment 6 (S6)	Handler (B) applies pressure on the lead rope and handler (C) encourages the donkeys across by an auditory sound. Handler C claps their hands from behind the donkey to make it move forward (Nansen and Blache, 2016). Pressure and sound are released or stopped when the donkey moves and reapplied when it stops until the donkey had completed the task.	A known frontal visual stimulus and an unknown rear acoustic stimulus.	Negative
Phase II. Response tests to object and sound recognition and association.			
Treatment 7 (S7)	Measured the donkeys' reaction towards the presence of the veterinarian when asked to complete the task.	Known visual and acoustic stimuli.	N/A
Treatments 8 and 9 (S8 and S9)	Measured the response of the donkeys to the sound of a horn. Handler (C) beeps a horn in front of the donkey once (Lanier et al., 2000). After that, handler (C) blares a horn in front of the donkey three times (Lanier et al., 2000).	Simultaneous unknown at first and later known frontal visual and acoustic stimuli.	N/A
Treatment 10 (S10)	A handler (C) played a car engine recording from a round red speaker in front of the donkey under study. All donkeys had previously been in contact with a car engine sound, but the stimulus came out of an unknown device.	Simultaneous unknown visual stimulus and a known acoustic stimulus.	N/A
Treatments 11 (S11) and 12 (S12)	Scored the reaction towards other donkeys in the same herd during all the tests and the reaction towards other species animals (cows, sheep, poultry, llamas, cats, and dogs) in the same farm to which the donkeys were accustomed.	Known visual and acoustic stimuli.	N/A

N/A: not applicable.

2.3. Problem-solving test and stimulus/treatment description

All behavioral responses were registered by only one trained judge during the annual behavior assessment sessions on four random days from June to November and from 2013 to 2015, as this is the period of time of the year during which the weather conditions are most consistent in the area where the study took place. Data were collected from 22 different farms in the Andalusian region of Spain.

The behavioral test used for this study consisted of two consecutive main phases that lasted for 15 min per animal on the whole, with no pause between the presentation of each of the consecutive treatments/stimuli. Time was evenly distributed throughout the consecution of the different treatments/stimuli. Each donkey was exposed to 12 external stimuli once. Phase I started when the animal was exposed to a 2 m^2

Table 3
Scale translation and description of the twelve mood or attitude reaction related adjectives considered and donkeys' response classification towards the twelve stimuli presented to them during the study.

Scale	Mood/Attitude	Description	Response	Scale	Degree/Intensity
1	Distracted	No reaction. Pays attention to other stimuli around.	Hyporeactive	1	Scored from 1 to 5
2	Depressive	No reaction. Pays reduced attention to it. Overall, body posture shows lowered head and neck, roundness to spine and tucked tail.	Hyporeactive	1	
3	Indifferent or nonresponsive	No reaction. Pays attention to it.	Hyporeactive	1	
4	Calm	Reaction, but stands still. Pays attention to other stimuli at the same time.	Neutral	2	
5	Awaiting	Reaction, but stands still. Only focuses on the stimulus presented.	Neutral	2	
6	Curious	Reaction. Pays attention and stands still moving its head towards the stimulus.	Neutral	2	
7	Cautious	Reaction. Pays attention and slightly moves towards the stimulus.	Neutral	2	
8	Mistrustful	Reaction. Pays attention and moves towards the stimulus slowly and doubtfully.	Neutral	2	
9	Surprised	Reaction. Only focused on the stimulus being presented. Gets startled but moves towards the stimulus calmly.	Hyperreactive	3	
10	Nervous	Reaction. Only focused on the stimulus being presented. Gets startled, and tries to move apart from it at first. Able to move towards it if led by the operator.	Hyperreactive	3	
11	Fearful	Reaction. Only focused on the stimulus being presented. Tries to move apart from it. Unable to move towards it if led by the operator.	Hyperreactive	3	
12	Rejection	Reaction. Only focused on the stimulus being presented. Gets startled, and moves apart from it noticeably. Pulls apart from the leading rope when the operator tries to move towards the stimulus.	Hyperreactive	3	

Table 4
Description of the thirteen traits comprising the intelligence and cognition clusters studied and definition of their scales in donkeys.

Trait	Definition	Scale	Description
Intelligence cluster			
Concentration	The animal collaborates during the assessment session and does not get distracted by the environment.	1	Distracted
		2	Poor
		3	Inconstant
		4	Intermediate
		5	Concentrated
Curiosity	The animal is interested in the novel stimuli being presented and moves towards them.	1	Never (0%)
		2	Rarely (5-10%)
		3	Sometimes (50%)
		4	Frequently (70%)
		5	Always (100%)
Memory	The animal remembers the stimuli being presented.	1	Scattered
		2	Poor short-term memory
		3	Average short-term memory
		4	Average long-term memory
		5	Good long-term memory
Stubbornness	The donkey rejects following the requests of the assessor.	1	Stubborn (Cautious)
		2	Indifferent
		3	Moaner
		4	Reluctant
		5	Obedient
Docility	The donkey easily follows the orders of the instructor.	1	Stubborn
		2	Indifferent
		3	Moaner
		4	Reluctant
		5	Obedient
Alertness	The animal shows a vigilant or alert status focusing on the stimulus around.	1	Untamed
		2	Unwilling
		3	Reticent
		4	Adaptable
		5	Docile
Cognition cluster			
Dependence	The donkey is comfortable when separated from the main herd	1	Dependent
		2	Restless
		3	Stable
		4	Adapted
		5	Calm
Trainability	Ability of the animal to be trained into the fulfillment of the tests	1	Never (0%)
		2	Rarely (5-10%)
		3	Sometimes (50%)
		4	Frequently (70%)
		5	Always (100%)
Cooperation	The donkey cooperates with its handlers during the daily tasks	1	Never (0%)
		2	Rarely (5-10%)
		3	Sometimes (50%)
		4	Frequently (70%)
		5	Always (100%)
Emotional stability	The animal is not predictable from one to another stimulus	1	Unpredictable
		2	Surprising
		3	Stable
		4	Balanced
		5	Predictable
Perseverance	The animal is patient when completing several sequential tests.	1	Inpatient
		2	Generally impatient but easily handled
		3	Patient but pushes the operator occasionally
		4	Patient without pushing the operator
		5	Awaits the operator's orders
Get in/out of stables	The animal shows no problem when leaving or entering its housing facilities.	1	Never (0%)
		2	Rarely (5-10%)
		3	Sometimes (50%)
		4	Frequently (70%)
		5	Always (100%)
Ease of handling	The animal shows sympathy towards humans.	1	Mistrustful towards humans in general
		2	Mistrustful towards unknown people
		3	Comfortable with familiar people, but mistrustful to unknown people
		4	Comfortable with the human presence
		5	Increased sympathy for human presence

oilcloth (vinyl fabric with a canvas-like cotton mesh backing featuring a wooden printed design) for the first time (novel object), and assessed the progressive response of the animals to stimuli one to six (Table 2), parallelly assessing the suitability of the use of negative, positive or lure reinforcement methods to effectively encourage donkeys to cross the oilcloth, to which they become progressively familiar, as the test continues (non-novel object).

The oilcloth was placed 2 m ahead in front of the donkey and relayed in the same position before testing every new animal. The response of the donkey was registered and quantified by the judge from the moment the oilcloth was relayed in front of the donkey by handler (A). Handler (B) was in charge of completing the task with the donkey by utilizing different treatments/stimuli (from one to six). Phase II assessed the response to treatments/stimuli seven to twelve (Table 2) and corresponds to the presentation of different acoustic or visual independent stimuli to the donkeys under study. The animals were videotaped (30 frames/s) at 2 m from the left side of the oilcloth, from the beginning of Phase I until the end of Phase II, for later further evaluation by the same person. The person videotaping the animals, was in charge of supervising each test followed the timing requirements mentioned above.

2.4. Cognitive process related traits definition and scales

Prior to the behavioral assessment, we conducted a telephone interview to survey the experience of the owners of the donkeys in the study to define the traits comprising the clusters to be considered in the model. First, we asked owners to identify the adjectives that they most commonly used to describe their donkeys' mood or attitude towards external stimuli. Among the answers that the respondents gave, we chose twelve adjectives as the most frequent ones to describe the response to external stimuli displayed to define the scales to assess the traits included in the coping style cluster (Table 3). We discarded the rest of adjectives because of the anecdotical occurrence of their use. This coping style cluster consisted of three scales. The first scale or mood/attitude scale translated the adjectives from the survey into a score ranging from 1 to 12, with increasing levels of arousal and evasive behavior. The second scale or response scale measured whether the donkeys were hyporeactive, neutral or hyperreactive, and ranged from 1 to 3, with one being hyporeactive, 2 meaning a neutral response and 3 describing a hyperreactive animal. We assigned a score number of 1 to highly hyporeactive or distracted donkeys, and a value of 12 to highly reactive or elusive donkeys moving apart from the stimuli. We used a third scale or degree/intensity scale to score the level at which each response in the mood/attitude scale was displayed from 1 to 5, with 1 meaning the lowest intensity response while a score of 5 describes the highest intensity response displayed. We simultaneously registered information on the relationship held with reinforcement techniques applied to educate donkeys on getting used to the novel stimuli presented (Table 2).

Secondly, we interviewed owners about their donkeys' inherent cognitive abilities, the tasks that they should routinely accomplish on their farms and the training/education methodology (or learning methods) owners regularly apply for their donkeys to learn such skills/ tasks. Among the answers the respondents gave, they coincided on thirteen traits which were chosen as they were the ones that the owners most frequently allude to during the interviews (Table 4). We discarded the rest of traits because of the anecdotical occurrence of their use or because of being related to the use of different nouns to allude the same behavioral trait concept.

We organized the information deriving from the interview for the thirteen behavioral traits in two clusters. A 'cognition' cluster comprising seven traits that were directly related to unspecific cognitive processes considering the ability of donkeys to perceive information from their environmental situation, and an 'intelligence' cluster comprising the six remaining traits, describing the cognitive processes or mental capacities of the donkeys to retain information from the environment as knowledge to be applied towards adaptive responses within a specific context. We translated these categorical traits into different linear scales, in which the donkeys scoring one meant they presented the lowest extreme behavioral pattern and five the highest extreme one. The thirteen intelligence and cognition related traits considered, and a detailed definition of the scores present in the scale is described in Table 4.

2.5. Statistical analyses

The present study initially considered sixteen traits comprising three main clusters according to the cluster definition context described above. Coping style cluster comprised three of these traits and was assessed separately due to the higher number of observations (n = 3 600) and factors involved, while the other two clusters 'cognition' (7 traits) and 'intelligence' (6 traits) were assessed together (n = 300), as they did not include the stimulus effect, which was non-significant ($P > 0.05$) for all the thirteen traits included in both clusters. To statistically support the organization of clusters initially described in the cluster definition context, we computed Pearson's correlations between the cognitive processes tested to ensure that none of them demonstrated very strong multicollinearity (> 0.95) what may suggest excluding those traits possibly measuring for the same cognitive process. Then, we performed an agglomerative hierarchical cluster analysis (HCA) using the centroid joining method with squared Euclidean distances to classify cognitive processes into groups with shared similarities to confirm the soundness of the *a priori* cognitive clustering division, by means of the Classify procedure from SPSS Statistics for Windows, Version 24.0, IBM Corp. (2016). The dependent variables measured (Tables 5 and 6) were of a continuous level and were assumed to be approximately normally distributed. The independent variables (year of assessment, husbandry system, sex and stimulus/treatment) each consisted of two or more categorical, independent groups with independence of observations and no significant outliers were found. We also assumed homogeneity of variances for each combination of the groups of the two independent variables, therefore, we performed a one-way ANOVA and a posthoc Tukey Test using the Means procedure from SPSS Statistics for Windows, Version 24.0, IBM Corp. (2016) to compute the fraction of the variance explained by each factor separately. Because of the small size of the sample, we used ε^2 and ω^2 to compute the effect size in the model, as they use unbiased measures of the variance components and report the least mean root square errors, therefore becoming suitable for behavioral studies (Okada, 2013), according to $\varepsilon^2 = \frac{SS_b - df_b MS_w}{SS_t}$ and $\omega^2 = \frac{SS_b - df_b MS_w}{SS_t + MS_w}$, respectively.

We performed a two-way MANOVA using the GLM procedure from SPSS Statistics for Windows, Version 24.0, IBM Corp. (2016) to compute the existing interactions between factors, as they are discontinuous variables. We used non-linear regression from SPSS Statistics for Windows, Version 24.0, IBM Corp. (2016) for two different statistic models consisting of three fixed effects; i.e.: assessment year (AY), 3 levels; sex (Sex), 2 levels and system (Sys), 5 levels and a covariate, age in months, and their separate repercussion on each of the sixteen variables. In the case of the coping style cluster, an additional effect comprising the stimuli (Sti) consisting of 12 levels was included (Table 2). The model fitted for the coping style cluster was:

$$Y = \mu + AY + Sex + Sys + Sti + AY^*Sex + AY^*Sys + AY^*Sti + Sex^*Sys + Sex^*Sti + Sys^*Sti + AY^*Sex^*Sys + AY^*Sex^*Sti + AY^*Sys^*Sti + AY^*Sex^*Sys^*St + A + \varepsilon$$

While the model for the intelligence and cognition clusters was:

$$Y = \mu + AY + Sex + Sys + AY^*Sex + AY^*Sys + Sex^*Sys + AY^*Sex^*Sys + A + \varepsilon$$

where,

Y = behavioral traits (1–16)
μ = mean
AY = assessment year (1–3)
Sex = sex (1, 2)

Table 5
Descriptive statistics for variables, fixed effects and covariables of coping style, intelligence and cognition related traits in Andalusian donkeys (n = 300).

Clusters	Effects	n	Minimum	Maximum	Mean	SEM	SD	CV
Fixed effects	Year	3 600	1	3	1.97	0.011	0.653	0.33
	System	3 600	1	5	2.58	0.016	0.971	0.25
	Stimulus	3 600	1	12	6.50	0.058	3.453	0.38
	Sex	3 600	1	2	1.74	0.007	0.439	0.53
Covariate	Age (in Months)	3 600	0.267	270.400	84.078	1.023	61.405	0.73
Coping style cluster	Response	3 600	1	3	2.26	0.008	0.473	0.21
	Mood	3 600	1	12	6.28	0.054	3.223	0.51
	Degree	3 600	1	5	3.28	0.026	1.534	0.47
Intelligence cluster	Concentration	300	1	5	3.80	0.059	1.027	0.27
	Curiosity	300	1	5	4.10	0.054	0.933	0.23
	Memory	300	1	5	4.11	0.060	1.035	0.25
	Stubbornness	300	1	5	3.67	0.068	1.174	0.32
	Docility	300	1	5	3.99	0.054	0.943	0.24
	Alertness	300	1	5	4.74	0.033	0.573	0.12
Cognition cluster	Dependence	300	1	5	4.33	0.063	1.089	0.25
	Trainability	300	1	5	3.80	0.060	1.035	0.27
	Cooperation	300	1	5	4.13	0.062	1.081	0.26
	Emotional stability	300	1	5	3.78	0.057	0.983	0.26
	Perseverance	300	1	5	4.64	0.044	0.762	0.16
	Get In/Out of Stables	300	1	5	4.58	0.046	0.791	0.17
	Ease of Handling	300	1	5	4.03	0.065	1.119	0.28

Table 6
Summary of the results of the ANOVA, posthoc Tukey Test and the determinative coefficient of the effect of each factor on weight through ε^2 and ω^2 estimators on the sixteen cognitive process-related traits assessed in Andalusian donkeys.

Cluster	Trait	Factors	F (df)[D]	P value	Levels (Mean)[C]	ε^2	ω^2
Coping styles							
Response		Year	26.088 (2)	0.000	2013 (2.36)^{bc} 2014 (2.23)^{a} 2015 (2.23)^{a}	0.0138	0.0137
		Sex	31.139 (1)	0.000	♂ (2.33) ♀ (2.23)	0.0083	0.0083
		System[A]	39.667 (4)	0.000	I (2.46)^{bcd} SI (2.16)^{ace} SE (2.26)^{abe} C (2.23)^{ae} E (2.42)^{bcd}	0.0412	0.0412
		Stimuli[B]	34.417 (11)	0.000	S1 (2.30)^{dgjkl} S2 (2.30)^{dgkl} S3 (2.21)^{dfgjkl} S4 (2.46)^{abceghikl} S5 (2.32)^{dgkl} S6 (2.36)^{cgkl} S7 (2.07)^{abcdefhij} S8 (2.33)^{dgkl} S9 (2.31)^{dgkl} S10 (2.42)^{acgkl} S11 (2.01)^{abcdefhij} S12 (2.01)^{abcdefhij}	0.0927	0.0926
Mood		Year	29.639 (2)	0.000	2013 (7.03)^{bc} 2014 (6.1)^{a} 2015 (5.94)^{a}	0.0157	0.0157
		Sex	23.089 (1)	0.000	♂ (6.71) ♀ (6.12)	0.0061	0.0061
		System[A]	40.534 (4)	0.000	I (7.65)^{bcd} SI (5.64)^{ace} SE (6.24)^{abe} C (6.19)^{ae} E (7.44)^{bcd}	0.0421	0.0421
		Stimuli[B]	62.107 (11)	0.000	S1 (6.91) ^{dgjkl} S2 (6.87)^{dgjkl} S3 (6.24)^{dfgjkl} S4 (7.98)^{abceghikl} S5 (6.82)^{dgjkl} S6 (7.19)^{cdghkl} S7 (4.54)^{abcdefhij} S8 (6.26)^{dfgjkl} S9 (6.58)^{dgjkl} S10 (7.8)^{abceghikl} S11 (4.08)^{abcdefhij} S12 (4.05)^{abcdefhij}	0.1574	0.1573
Degree		Year	57.152 (2)	0.000	2013 (3.00)^{b} 2014 (3.52)^{ac} 2015 (2.94)^{b}	0.0303	0.0303
		Sex	13.899 (1)	0.000	♂ (3.12) ♀ (3.34)	0.0036	0.0036
		System[A]	55.021 (4)	0.000	I (2.95)^{bc} SI (3.76)^{ace} SE (3.05)^{bd} C (3.53)^{ace} E (2.71)^{bd}	0.0566	0.0566
		Stimuli[B]	45.763 (11)	0.000	S1 (2.56)^{ceghijkl} S2 (2.81)^{ghijkl} S3 (3.15)^{adghij} S4 (2.55)^{ceghijkl} S5 (3.1)^{adghij} S6 (2.78)^{ghijkl} S7 (3.82)^{abcdefjkl} S8 (3.66)^{abcdefj} S9 (3.96)^{abcdefkl} S10 (4.27)^{abcdefghkl} S11 (3.39)^{abdfgij} S12 (3.37)^{abdfgij}	0.1203	0.1203
Cognition							
Dependence		Year	8.817 (2)	0.000	2013 (3.29)^{c} 2014 (4.03)^{c} 2015 (3.62)^{ab}	0.0937	0.0934
		Sex	2.022 (1)	0.156	♂ (3.62) ♀ (3.84)	0.0070	0.0069
		System[A]	5.584 (4)	0.000	I (3.46) SI (4.05)^{ce} SE (3.65)^{b} C (4.18)^{e} E (3.53)^{bd}	0.0468	0.0467
Trainability		Year	3.850 (2)	0.022	2013 (3.34)^{b} 2014 (3.84)^{a} 2015 (3.57)	0.0257	0.0256
		Sex	1.665 (1)	0.198	♂ (3.58) ♀ (3.71)	0.0000	0.0000
		System[A]	3.987 (4)	0.004	I (3.00)^{b} SI (4)^{a} SE (3.65) C (3.59) E (3.47)	0.0567	0.0566
Cooperation		Year	8.067 (2)	0.000	2013 (3.74)^{b} 2014 (4.12)^{ac} 2015 (3.93)^{b}	0.0211	0.0210
		Sex	3.776 (1)	0.053	♂ (3.82) ♀ (4.05)	0.0085	0.0085
		System[A]	10.723 (4)	0.000	I (3.76)^{bc} SI (4.25)^{acde} SE (3.96)^{ab} C (3.82)^{b} E (3.35)^{b}	0.0499	0.0497
Emotional stability		Year	16.458 (2)	0.000	2013 (4.57)^{b} 2014 (4.73)^{ac} 2015 (4.95)^{b}	0.0400	0.0399
		Sex	3.099 (1)	0.079	♂ (4.9) ♀ (4.68)	0.0245	0.0244
		System[A]	4.672 (4)	0.001	I (4.86)^{b} SI (4.74)^{ac} SE (4.72)^{b} C (4.82) E (4.53)	0.0021	0.0021
Perseverance		Year	5.054 (2)	0.007	2013 (4.40)^{b} 2014 (4.74)^{a} 2015 (4.62)	0.0264	0.0263
		Sex	0.648 (1)	0.421	♂ (4.58) ♀ (4.66)	0.0000	0.0000
		System[A]	2.130 (4)	0.077	I (4.62) SI (4.8) SE (4.54) C (4.59) E (4.41)	0.0149	0.0149
Get In/Out of Stables		Year	13.800 (2)	0.000	2013 (4.26)^{b} 2014 (4.78)^{ac} 2015 (4.38)^{b}	0.0789	0.0786
		Sex	7.715 (1)	0.006	♂ (4.37) ♀ (4.66)	0.0220	0.0219
		System[A]	7.786 (4)	0.000	I (4.78)^{d} SI (4.85)^{cde} SE (4.41)^{b} C (4.12)^{ab} E (4.29)^{b}	0.0832	0.0830
Ease of Handling		Year	8.028 (2)	0.000	2013 (3.59)^{b} 2014 (4.22)^{a} 2015 (4.00)	0.0449	0.0448
		Sex	3.725 (1)	0.055	♂ (3.82) ♀ (4.10)	0.0090	0.0090
		System[A]	8.395 (4)	0.000	I (3.41)^{bc} SI (4.39)^{ae} SE (4.06)^{ae} C (3.76) E (3.29)^{bc}	0.0900	0.0898
Intelligence							
Concentration		Year	3.218 (2)	0.041	2013 (3.53)^{b} 2014 (3.90)^{a} 2015 (3.85)	0.0146	0.0146
		Sex	5.811 (1)	0.017	♂ (3.56) ♀ (3.89)	0.0158	0.0158
		System[A]	5.434 (4)	0.000	I (3.38)^{b} SI (4.13)^{ace} SE (3.72)^{b} C (3.82) E (3.35)^{b}	0.0560	0.0558
Curiosity		Year	3.997 (2)	0.019	2013^{bc} (4.43) 2014 (4.47)^{a} 2015 (3.82)^{a}	0.0497	0.0495
		Sex	0.610 (1)	0.435	♂ (4.18) ♀ (4.38)	0.0034	0.0034
		System[A]	2.809 (4)	0.026	I (4.08) SI (4.64)^{ce} SE (4.17)^{b} C (4.82)^{e} E (3.76)^{bd}	0.0578	0.0576
Memory		Year	15.276	0.000	2013 (3.50)^{bc} 2014 (3.91)^{a} 2015 (3.82)^{a}	0.0187	0.0186
		Sex	1.570 (1)	0.211	♂ (3.67) ♀ (3.84)	0.0022	0.0022
		System[A]	12.015 (4)	0.000	I (3.35)^{bcd} SI (4.05)^{ace} SE (3.77)^{ab} C (3.82)^{ae} E (3.41)^{bd}	0.0384	0.0383
Stubbornness		Year	4.943 (2)	0.008	2013 (3.82)^{b} 2014 (4.16)^{a} 2015 (4.23)	0.0197	0.0196
		Sex	0.710 (1)	0.400	♂ (4.03) ♀ (4.12)	0.0000	0.0000
		System[A]	5.497 (4)	0.000	I (3.68)^{bc} SI (4.17)^{a} SE (4.1)^{a} C (4.47) E (4.18)	0.0236	0.0235
Docility		Year	4.216 (2)	0.016	2013 (3.54)^{b} 2014 (4.33)^{a} 2015 (4.15)	0.0872	0.0869
		Sex	3.569 (1)	0.060	♂ (3.99) ♀ (4.16)	0.0019	0.0019
		System[A]	4.924 (4)	0.001	I (3.43)^{b} SI (4.53)^{ae} SE (4.04) C (4.35) E (3.41)^{b}	0.1284	0.1281
Alertness		Year	7.227 (2)	0.001	2013 (3.76)^{c} 2014 (4.33)^{c} 2015 (3.95)^{ab}	0.0451	0.0450
		Sex	8.504 (1)	0.004	♂ (3.92) ♀ (4.20)	0.0092	0.0092
		System[A]	1.158 (4)	0.329	I (3.46) SI (4.59) SE (4.06) C (3.82) E (3.65)	0.1151	0.1148

[A] Husbandry system classification: I (Intensive), SI (Semi intensive), SE (Semi extensive), C (Contest), E (Extensive).
[B] From S1 to S12, these are the stimuli to which the donkeys were exposed.
[C] Superindexes denote the levels of the traits among which there was a statistically significant difference $P < 0.05$. Levels: Year (^{a}2013, ^{b}2014, ^{c}2015); System (^{a}I, ^{b}SI, ^{c}SE, ^{d}C, ^{e}E); Stimuli (S1^{a}, S2^{b}, S3^{c}, S4^{d}, S5^{e}, S6^{f}, S7^{g}, S8^{h}, S9^{i}, S10^{j}, S11^{k}, S12^{l}).
[D] F(df): Snedecor's F (degrees of freedom).

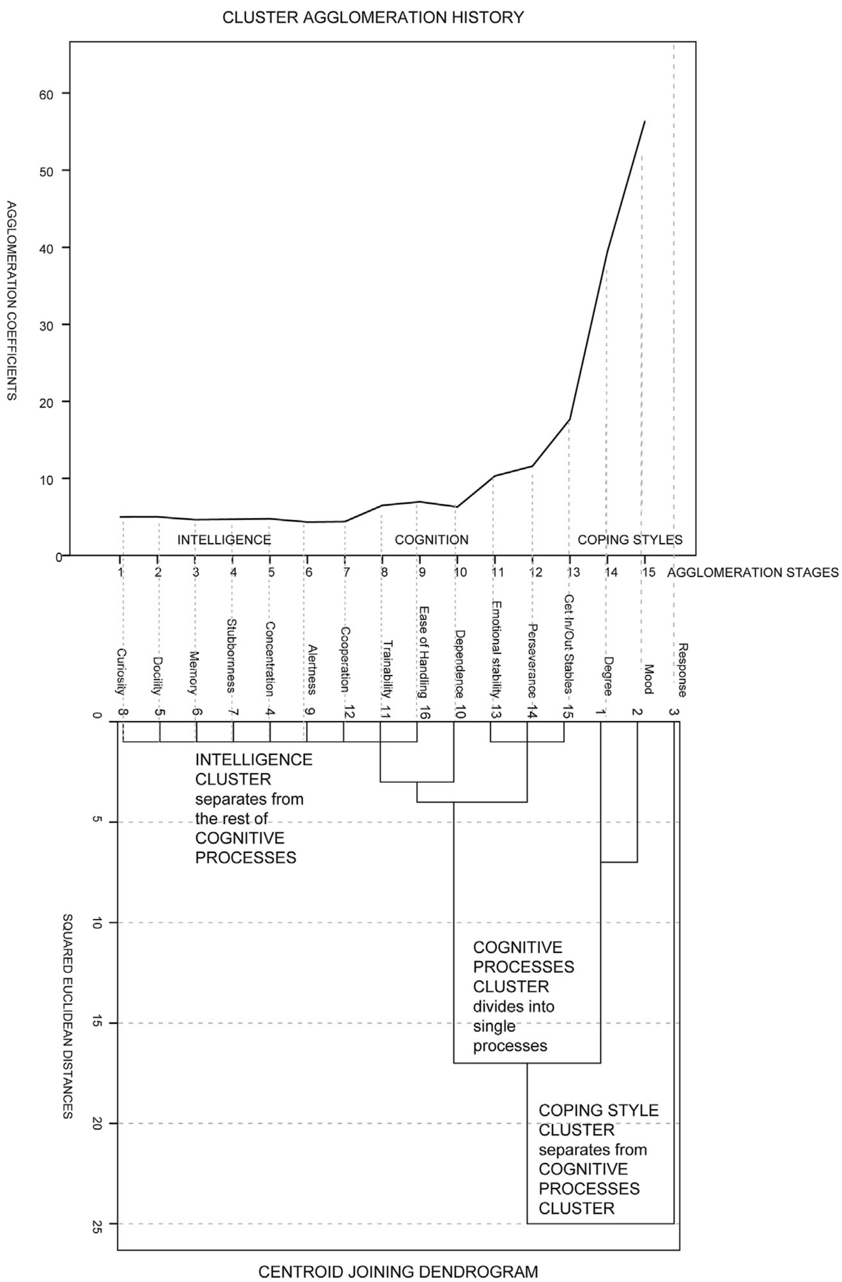

Fig. 1. Hierarchical cluster agglomeration history and centroid joining cluster dendrogram representation of the three clusters (coping styles, cognition and intelligence) comprising the sixteen cognitive processes related variables in the study.

Sys = system (1–5)
Sti = stimulus (1–12)

$$AY*Sex + AY*Sys + AY*Sti + Sex*Sys + Sex*Sti + Sys*Sti + AY*Sex*Sys + AY*Sex*Sti + AY*Sys*Sti + AY*Sex*Sys*Sti$$

= interaction between several levels

A = age (months)
ε = residual error.

We used the age of the donkeys expressed in months as a linear and quadratic covariate to correct the phenotype observation of each behavioral variable. The reason for this inclusion is the fact that despite all donkeys were not born on the same day, they were scored together, so that assessed at different ages. We could expect the residual error of the model to be remarkably important given the increased likelihood of the existence of factors influencing the cognitive processes assessed that may not be controlled by the model, one of the main drawbacks when studying behavioral genetics.

3. Results

The three clusters initially set according to bibliography matched the results obtained for the preliminary HCA. HCA variable distribution and agglomeration coefficients and stages are shown in Fig. 1. The Pearson's correlations among all cognitive processes highlighted the individuality of the cognitive processes studied, ranged from − 0.084 to 0.812 and were highly statistically significant ($P < 0.001$) except for the alertness process, whose correlations were statistically significant ($P < 0.05$) for stubbornness, dependence, cooperation and emotional stability and were not significant for all the variables in the coping style cluster. A summary of the results of the descriptive statistics analysis is shown in Table 5. A summary of the main results of the one-way ANOVA, posthoc Tukey Test and effect size estimator, ε^2 and ω^2 is shown in Table 6. A summary of the determinative coefficients of the significant levels of factors, interactions, covariates and models obtained with MANOVA for all behavioral traits is shown in Tables 7 and 8.

Research in Veterinary Science 113 (2017) 105–114

Table 7
Signification (P values) and determinative coefficients (reduced or adjusted R^2) for each possible double and multiple factor interaction, covariates and models obtained with MANOVA for coping style cognitive process related traits in Andalusian donkeys.

Effects/traits	Response	Mood	Degree
Age (in months)	0.000	0.000	0.000
Year * sex	0.024	0.010	0.081
Year * system	0.000	0.000	0.000
Year * stimulus	0.023	0.000	0.996
Sex * system	0.000	0.000	0.000
Sex * stimulus	0.499	0.755	0.788
System * stimulus	0.000	0.000	0.000
Year * sex * system	0.000	0.000	0.010
Year * sex * stimulus	0.963	0.989	0.695
Year * system * stimulus	0.335	0.217	0.012
Sex * system * stimulus	0.079	0.066	0.852
Year * sex * system * stimulus	0.644	0.956	0.991
Reduced R^2	0.243	0.328	0.346

4. Discussion

The selection and registration of Andalusian donkeys occurs at the age of 3 years old, similarly to what happens in some horse breeds such as the Hanoverian, Dutch and Swedish Warmblood, Selle Français and Irish Sports Horse (Thorén Hellsten et al., 2006), when the individuals are assessed and included in or excluded from the studbook of the breed. This selective process has traditionally emphasized on the adherence of the individuals to morphological and phaneroptical standards exclusively.

The worldwide endangerment status of donkey breeds contrasts their potential new functional niches. This situation promotes research development to adapt the traditional standards to such new functional perspectives. Systematic data collection and genetic evaluation for functional traits may provide breeders with more objective tools when selecting their breeding stock to enhance selection response (Arnason and Van Vleck, 2000).

Organized breeding programmes have proved to be effective for other more profitable species like horses. At this point, the possibility of harmonizing selection programmes across different countries setting the same breeding objectives has been suggested as an interesting measure for the development of breeds.

However, the definition of donkey breeding objectives is not an easy task to accomplish as no selection has been carried out yet on this species. Therefore, there is no clue about which traits should be taken into account, nor which non-genetic factors should be controlled to face the new functional perspectives. In species such as the donkey, in which their functional roles are so closely related to humans, behavior becomes a key element to consider.

The quantitative study of behavior and especially cognitive processes, often deals with overlapping processes. To categorize such processes, we tested donkeys for their responses in a standardized test to prevent the behavioral traits assessed from containing elements of other distorting behavioral elements such as reactions to social separation.

Although we expected the statistical analysis to report some high Pearson's correlations because of the similar nature of the cognitive processes measured, we did not detect potential redundancies among processes (all Pearson's correlations ≤ 0.812). The results of the Centroid hierarchical cluster analysis successfully matched our previous cluster definition hypothesis as it organized the sixteen traits studied into the three clusters (Fig. 1). This analysis proceeds from each cognitive process constituting its own cluster, to all of the processes being iteratively and progressively combined into a single global cluster (Jarvis et al., 2003; Norušis, 2012). Then, we selected the iteration that best represented the three clusters that we had previously determined by examining the agglomeration stages and coefficients obtained (Fig. 1).

The study of behavior especially faces compromises when we try to define the terms involved in specific studies. These difficulties may be mainly ascribed to the existing inconsistency across situations because of the lack of accurate descriptions of the traits being studied or to the lack of a common training of the observers.

Age adds an additional difficulty as personality and cognition in humans (Soubelet and Salthouse, 2011) and equids (Wolff and Hausberger, 1994) seems to interconnectedly evolve in time, especially when considering which responses are presented and at which degree they are performed at different ages. The mean age of the donkeys at evaluation was 84.08 months, with a coefficient of variation (CV) of above 73% (Table 5). Because of the fact that behavioral processes are the result of a dynamic interaction between the genetic background and environmental factors such as previous experiences (Boissy et al., 2005), age may affect the result obtained. For instance, the study by Oki et al. (2007) generally considered young horses comparing to the heterogeneity of the age range in our present study, what may have affected our results. Age factor resulted highly significant ($P < 0.001$) except for the alertness trait included in the intelligence cluster (Table 5).

The fixed effects that comprise our model were chosen after performing a thorough bibliographic review on equine behavior and the factors significantly affecting it (Hausberger and Muller, 2002). Among the factors that influence equine behavior, sex and environment as described by Hausberger et al. (2004) or French (1993) and body condition (McCall, 1989) have generally proved to present a strong effect on equine behavioral traits. The rest of fixed effects controlled in our study consisted of the year (a 3-year period from 2013, 2014 and 2015), and the 12 stimuli presented and used to score the behavioral responses displayed (Table 2).

Table 8
Signification (P values) and determinative coefficients (reduced or adjusted R^2) for each possible double and multiple factor interaction, covariates and models obtained with MANOVA for intelligence and cognition traits in Andalusian donkeys.

Effects/traits	Age (in months)	Sex * year	Sex * system	Year * system	Sex * year * system	Reduced R^2
Concentration	0.000	0.453	0.430	0.375	0.335	0.161
Dependence	0.000	0.139	0.109	0.023	0.886	0.276
Trainability	0.000	0.074	0.516	0.645	0.716	0.202
Curiosity	0.000	0.130	0.073	0.889	0.465	0.143
Memory	0.000	0.718	0.099	0.034	0.550	0.372
Cooperation	0.000	0.413	0.080	0.316	0.592	0.311
Emotional stability	0.000	0.162	0.495	0.664	0.601	0.291
Stubbornness	0.000	0.260	0.427	0.448	0.368	0.198
Docility	0.000	0.001	0.352	0.785	0.113	0.233
Alertness	0.110	0.189	0.418	0.003	0.174	0.194
Perseverance	0.000	0.091	0.683	0.256	0.787	0.110
Get in/out of stables	0.000	0.280	0.702	0.000	0.391	0.286
Ease ot handling	0.000	0.050	0.533	0.394	0.665	0.297

The sample analyzed was unequally distributed in 22 farms all over Andalusia. Traditionally, one to three animals is kept on the farms in which breeding is not one of the primary productive objectives and locations gathering a higher number of individuals are anecdotical. This context made a farm/herd effect not to be considered, as the 40.91% of the 22 farms involved in this study only housed from 1 to 3 donkeys. With almost half of the farms accounting for only 12 animals from the sample, computing a herd effect would distort the results, hindering the estimation of the farm variation source. To overcome this difficulty, common farm characteristics were assessed to classify them into different husbandry systems, which helped to reduce such potential distortion.

Several specific studies in donkeys and other equids have reported the relevance of environment and handling on behavior patterns (French, 1993; Keeling et al., 2016; Lansade et al., 2004). All these factors are gathered in the husbandry system fixed effect that comprises 5 levels: Intensive, semi-intensive, semi-extensive, contest and extensive. The intensive level describes intensive farms in which the donkeys normally live in boxes or other reduced space facilities, but what is more important, in which the donkey contact with humans occurs on a daily basis, and which are frequently handled for more than just the minimum routinely hygienical and sanitary inspection tasks. The semi-intensive level describes farms in which the donkeys, apart from the previously described characteristics for the intensive level, are left roam in wider territory extensions but still keeping the daily contact basis with the people in their charge. This human contact time situation inverts in the semi-extensive level in which donkeys are kept in wider extensions and with whom the human contact is not kept daily, although the donkeys are still familiar and respond to the owner's requests. The contest level alludes to situations in which the animals are assessed under conditions that they are not accustomed to (Official Morphological Contest of the Breed), as the donkeys are transported to different facilities to theirs, and therefore they do not maintain the same human contact basis, nor they are surrounded by their home environment. Last, the extensive level gathers farms in which the contact with humans only occurs when sanitary inspection actions, vaccination campaigns or microchipping sessions are carried out or when the donkeys are being evaluated for their inclusion in the breed studbook, to then be left into a totally extensive nearly semi feral status.

The effect of the husbandry system was highly significant ($P < 0.001$) on all the behavioral traits assessed.

The effect of sires on the behavioral responses developed by their offspring has been highlighted by authors such as Hausberger et al. (2004) who reported a statistically significant effect. This is not surprising as the additive genetical component of behavioral traits imply both a sire and a dam effect on the traits assessed and therefore, both progenitors are half relevant when configuring the breeding value of a certain animal and not just the sire. The interaction of sex with behavioral traits has also been suggested in horses (Wolff and Hausberger, 1996) so that the model in our study included it as a fixed effect. Sexual dimorphism was evident in the breed for six of the sixteen traits. All the traits in the coping style cluster and concentration, alertness and the ability to get in or out stables (from the cognition and intelligence clusters) were significantly different between males and females ($P < 0.05$) as has been addressed in Table 6.

Only a few double and multiple interactions between the four factors controlled in the 'coping style' model are non-significant ($P > 0.05$) for the response, mood and degree traits, while the most of the double and triple interactions are significant ($P < 0.05$) for the three variables under study (Table 5). In the case of the 'intelligence' and 'cognition' model the interactions between the three factors involved were non-significant as well for all the thirteen traits studied. Double interactions between system and year were not significant ($P > 0.05$) except for dependence, memory, alertness and the ease of getting out or in stables, while the double interaction between system and sex was not significant ($P > 0.05$) for all traits (Table 5). ε^2 and ω^2 determinative coefficients for each trait assessed ranged between 15.74% and 0.19%.

Similar linear scales aiming at assessing behavioral traits and specifically coping style traits have similarly been studied in horses (Calviello et al., 2016), though the studies have not deepened or divided the components to study them separately and no genetical inference has been made yet.

The nature of the system that is currently used to evaluate the individuals being recognized as pure Andalusian breed donkeys could lead to an increase in environmental variability, considering the subjectivity inherently attached to the judgment of traits such as behavior (even though the judges are trained and experienced). The adoption of a linear scoring system in which the traits are evaluated in a continuous scale corresponding to the expression of cognitive or other behavioral process-related traits between two biological extremes may result in much better distribution properties enabling a better quantification of the traits measured (Rustin et al., 2009).

The Andalusian donkey breeding programme has resulted in the moderate genetic improvement of conformation, type, and phaneroptical traits, but some adjustment and refinement can be introduced to optimize selection responses. The formal definition of the breeding objectives is the key element of any genetic improvement programme (Van Vleck, 1993), and in the case of the Andalusian donkey, the need to include functional traits in the breeding goals, while maintaining selection for morphological and type characteristics, is essential.

The next step is to assess the information provided by these behavioral tests and to seek for genetic parameters when expanding the information and comparing it together with the genealogical data of the pedigree to implement a systematic genetic evaluation procedure, allowing the objective and early selection of breeding animals. An initial genetic assessment reported a mean heritability value of 0.20 ± 0.021 for the coping style cluster, 0.18 ± 0.13 for the cognition cluster and 0.21 ± 0.14 for the intelligence cluster, respectively, which will be studied and discussed in future studies. Simultaneously, the breeding programme can be further optimized by reducing generation intervals (through the registration of behavioral responses systematically at an earlier age and genetic evaluation of young animals).

This study sets the basis for behavioral traits to be considered as new selection criteria, hence, large studies carried out over several years and containing a higher number of animals is needed before any precise measures concerning the influence of the genetic and environmental effects can be determined. Nonetheless, selection for better-behaved donkeys would be potentially beneficial for donkeys' welfare and to reduce the number of accidents related to equestrian activities, as well as for the analysis of their suitability for assisted therapy programmes or any other human-related activity.

5. Conclusions

Statistical univariate and multivariate models can help isolate the effect of different variation factors on certain behavioral traits. The determinative coefficient for each of these factors becomes then an indicator of the fraction of the variation that such factors explain. The difficulty to find and control models to assess animal behavior especially increases when we intend to do it under field practical situations. The levels of significance found, show that the model used to assess the coping style cluster is more accurate and suitable than the one used to test for intelligence and cognition traits. This situation not only enables a more objective quantification methodology for coping styles related traits but also reports more reliable global results. The differences appearing because of the influence of the different fixed effects on the behavioral traits assessed may be attributed to the fact that the tests used may, in fact, evaluate the ability of certain owners to educate their donkeys rather than the inner cognitive capacity of the animals to develop a certain process. Although sexual dimorphism is evident on some of the cognitive processes, the variation may be ascribed to differences in the handling methods and routines applied to jacks and jennies. The husbandry system applied can help us group the animals to save the potential result distortion that may occur due to the unequal distribution of animals among the farms. The fraction of variance explained by external factors may be low when we considered them individually, but it can improve when their partial weights are summarized. The variance explained by these multifactorial models permits comparatively considering them to be efficient to quantify the sixteen cognitive processes in our study, as they provide very useful information for the design and ease of the complex models used in behavioral genetic analyses. Both double and triple interactions were mostly non-significant for intelligence and cognition clusters. This finding supports the fact that, in behavioral studies, the reliance on several factors individually, may help us quantify the factors or effects involved more accurately than their conjoint effects. Our results suggest the suitability of the proposed cognitive recording system to be applied in the routinely genetic selection of donkeys. These breeding criteria will be implemented in the future in order to make the donkey more commercially competitive and useful, not only aiming at saving animals but whole breeds from extinction.

Welfare declaration

All farms included in the study followed specific codes of good practices for equids and particularly donkeys and therefore, the animals received humane care in compliance with the national guide for the care and use of laboratory and farm animals in research, receiving the approval from local and regional Welfare Committees.

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