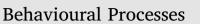
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Genetic parameter and breeding value estimation of donkeys' problemfocused coping styles

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ABSTRACT

Donkeys are recognized therapy or leisure-riding animals. Anecdotal evidence has suggested that more reactive donkeys or those more easily engaging flight mechanisms tend to be easier to train compared to those displaying the natural donkey behaviour of fight. This context brings together the need to quantify such traits and to genetically select donkeys displaying a neutral reaction during training, because of its implication with handler/rider safety and trainability. We analysed the scores for coping style traits from 300 Andalusian donkeys from 2013 to 2015. Three scales were applied to describe donkeys' response to 12 stimuli. Genetic parameters were estimated using multivariate models with year, sex, husbandry system and stimulus as fixed effects and age as a linear and quadratic covariable. Heritabilities were moderate, 0.18 ± 0.020 to 0.21 ± 0.021 . Phenotypic correlations between intensity and mood/emotion or response type were negative and moderate (-0.21 and -0.25, respectively). Genetic correlations between the same variables were mode/emotion and response type were positive and high (0.92 and 0.95, respectively). Breeding values enable selection methods that could lead to endangered breed preservation and genetically selecting donkeys for the uses that they may be most suitable.

1. Introduction

In psychology, coping refers to the conscious efforts of an individual to solve personal and interpersonal problems in order to master, minimize or tolerate stress (Weiten and Lloyd 2008). Coping mechanisms are commonly termed coping strategies, and they normally comprise adaptive strategies or strategies which reduce stress (Lazarus and Folkman, 1984). Benus et al. (1991) rodent experiments concluded that the response to external stimuli could mainly be classified into two equally valuable strategy alternatives to face daily environmental demands, passive and active animals. Koolhaas et al. (1999), suggested updating these 'styles' to proactive and reactive, as the former confusing terms did not consider fundamental differences. One of such fundamental differences is the degree in which behaviour is influenced by environmental stimuli. To sum up, the performance of routine rather intrinsically driven rigid types of behaviour found in proactive animals,

contrasts the generally more flexible and reactive attitude to environmental stimuli of reactive animals. Thus, when we speak about coping, we generally refer to reactive coping or the coping response after the presentation of the stressor. This differs from proactive coping, in which a coping response aims to neutralize a future stressor. Rather subconscious or non-conscious strategies such as defence mechanisms are generally excluded from the field of coping (Kramer, 2010).

The effectiveness of the coping effort depends on the type of stressful stimulus, the individual, and the circumstances. Coping responses are partly controlled by personality and mood, but also partly by the stressful nature of the environment around (Carver and Connor-Smith, 2010).

Among the four strategies that Weiten and Lloyd (2008) identified as coping styles in humans, problem-focused coping styles address those adaptive behavioral responses aimed at reducing, adapting or eliminating stressors. Although equids' reactiveness could clearly fit within

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these coping styles, a remarkable dimorphism has been described among species. Some equids describe a rather reactive strategy or tendency to freeze (such as donkeys) when they are involved in a challenging situation while others proactively flee, i.e. zebras or horses (Weaver, 2008).

Domesticated donkeys' wild ancestors often lived solitarily or in verv small groups of two animals in which running away was not always such a successful survival method compared to that of the horse that lives in larger hierarchical groups and forms stronger bonds with its congeners (Proops et al., 2012; The Donkey Sanctuary, 2014). Conversely, wild or even feral donkeys' close bonds remain more solitary, normally being established between the jenny and its foal. When facing a potentially threating stimulus, donkeys may display to the predator (or observer) apparently normal behavioural patterns. However, these "normal" behavioural patterns could also be associated with misunderstood negative affective states (Moehlman, 1998). Apart from clear psychological differences, which may have an ancestral social basis, Koolhaas and Bohus (1989) suggest that each of these strategies may be catalyzed by different endocrine responses. These endocrine responses may be the basis and therefore, influence the mechanisms adopted by animals to maintain control over potentially threatening situations.

Most of human-equid accidents result from unexpected animal reactions (Keeling et al., 1999). Daily human-animal interaction helps deepening the mutual interspecific bonds that are established (that is, improves the familiarity of the animals towards their handlers). These concepts have been suggested to be the basis for a better performance when obtaining neutrally responding individuals in very evolutionarily distant species (Simianer and Köhn 2010; Cibulski et al., 2014). Training processes can be conducted following different approaches. Thus, although a greater difficulty training certain donkeys may reduce their working life and increase the time and costs needed to obtain fully functional animals, this should not make us exclude such animals from their use in riding or therapy (Batt et al., 2008), but to tailor a different approach to educate them.

Methodology to select for coping styles or reactivity levels may be useful for breeders and owners. Identifying the coping styles displayed by donkeys or their reactivity level when facing diverse kinds of stimuli from the beginning enables appropriate training protocols to be implemented from day one to work with the animal's innate response and tailor training programmes to meet the animal's needs. Such implications and knowledge may improve their final destination to develop the tasks that they may be better fit for.

Meta-analytic studies of the fixed or random effects to be considered in genetic models become particularly necessary in behavioural genetics (Navas et al., 2017a). These effects may present small effect sizes on particular traits; however, they may still be statistically significant. In unison, these effects can explain quite a large proportion of the phenotypic variation for the traits studied in a population, hence, conditioning the estimates for genetic parameters of such traits.

The higher the determinative coefficient (R^2) in a general linear model is, the lower the residual variance unexplained by the effects that we have controlled in our model will be. Among other determinative coefficients, ϵ^2 and ω^2 use unbiased measures of the variance components and report the least mean root square errors, therefore becoming suitable for behavioral studies with a large number of effects involved (Okada, 2013).

In genetic analyses, the variation for a certain trait in a population, depends on the number of animals that represent each of the possible combinations among the effects affecting a certain trait included in the model testing for such trait. When our sample is so small that it lacks a high enough fraction of animals representing each of these possible combinations, the model turns invalid to measure for the trait that it was aimed at measuring. That is, this model may misrepresent the real biological variation found for that trait in particular in the population under study, considering all the possible combinations of effects involved.

We should carefully consider which effects represent mere experimental design effects and which of them are biologically relevant for our trait and should therefore be included in our genetic models.

Limited research has studied the genetic background of coping styles or reactivity in horses and none has focused on studying such traits in donkeys. Oki et al. (2007) estimated the heritability of behavioural responses at veterinary inspections for three consecutive years and found highly repeatable (0.97–0.98) heritabilities (0.23–0.28). The lower limit for horses' heritability of reactivity in literature is 0.17, reported by Rothmann et al. (2014). However, the accuracy of the heritability reported by these authors was low, probably because to the low number of horses in the study. Therefore, the aim of this study is first, to describe a model to compute the effects influencing response type, mood and response intensity to isolate the genetic background behind coping strategies in donkeys. Second, to estimate the genetic parameters for the three above-mentioned variables aiming at outlining the possibly existing overlapping among the behavioural variables tested. Third, to assess the genetic and phenotypic correlations of the coping styles or reactivity patterns expressed by donkeys when facing visual and auditory stimuli. Fourth, the development of an index addressing the possibility to genetically select for hyporeactive, neutrally responsive and hyperreactive animals suggesting the possible inclusion of these traits as breeding programme selection criteria.

2. Material and methods

2.1. Animal sample and study background

Direct records included the information from 300 Andalusian breed donkeys, 78 jacks and 222 jennies. As age range was not normally distributed (P < 0.05 for both Kolmogorov-Smirnov and Shapiro-Wilk's tests for normality) we used minimum, Q1, median, O3 and maximum to describe the age range in our sample. Minimum age in the range was 0.27 months, Q1 age was 29.76 months, median age was 77.04 months, Q₃ age was 129.07 months and maximum age was 270.40 months. Such wide age range was considered, as the stimuli battery used to test for coping styles/reactivity was suitable for all animals included in the study and given the fact that we assess an endangered breed from which the information belonging to each individual is indispensable. The donkeys in the sample were the progeny of 93 jackstocks and 253 jennies. All the donkeys were registered in the breeds' Spanish studbook. Their pedigree is routinely genetically tested through microsatellite-assisted genotyping and parentage tests for the resulting offspring of each mating.

2.2. Behavioural tests, scales and phenotyping

The records were measured during the yearly behaviour assessment sessions carried out over four randomly chosen days from June to November per year from 2013 to 2015 at twenty-two different farms all over Andalusia (southern Spain). Such sessions were developed to fulfil the requirements of the Order of 22nd September 2011, establishing the regulatory bases for the concession of grants to officially recognized entities for the management of the studbooks of livestock breeds for the conservation of livestock resources in the framework of the programme of Rural development of Andalusia 2007-2013. Each record comprised 3 scores for each animal which described the coping strategies that the animals developed when they were made face twelve consecutive stimuli which combined different elements (people, animals or objects) (Fig. 2). These elements could be unknown (the animal had not been familiarized with them) or known (the animal had already been familiarized), visual (could be perceived with the eyes, i.e. all of the stimuli) or visual and acoustic (apart from being perceived with the eyes, they generated sounds, i.e., a horn and a red speaker to play a car engine playback). These elements were presented to the donkeys from

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