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Characterization of social behavior in a group of domestic donkeys (*Equus asinus*)

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A B S T R A C T

Although the popularity of domestic donkeys is increasing, the scientific literature on their behavior is still limited. This study investigated the social behavior of a group of 13 donkeys (10 females, 3 geldings, aged 3–13 years) stabled in an Animal Assisted Interventions facility in the North of Italy. Our first aim was to assess the distribution of donkeys' intraspecific social behaviors during daytime and the second aim was to characterize affiliative and agonistic social interactions during peak activity times. Social behaviors were expressed more between 8 and 9 AM than the rest of daytime, mainly reflecting the expression of affiliative behavior. The latter consisted mostly of proximity (55.0% of all affiliative) and following (29.2%). In most cases affiliative behavior was expressed within preferential dyads, composed of either a mother and her offspring or two adult donkeys. This supports the idea that dyadic relationships are a predominant feature of the social organization of the species. However, dyadic interactions were not observed in the oldest sub-adults, suggesting that social preferences undergo a change around the time donkeys reach full growth. Agonistic behaviors were much less common than affiliative ones. The most expressed agonistic behavior was threat (51%), followed by displacement and bite (both 18.5%). They were less expressed by donkeys who were part of an affiliative pair, suggesting that limited expression of agonistic behavior is associated with an actual change in social preferences, not a mere consequence of fewer occasions of conflict over resources. While the study has limitations, the results provide insight into the social behavior of domestic donkeys which could represent a starting point for further research as well as relevant information for donkeys farming.

1. Introduction

The donkey population in the southern states of the European Union has decreased markedly in the second half of the twentieth century. In Italy, in particular, populations decreased by more than 95% between 1939 and 1996 (Starkey and Starkey, 2000). Recently, in many Mediterranean Countries, the interest in keeping and breeding donkeys has increased again, due to meat and milk production (Camillo et al., 2018). Donkey milk is considered to be a pharma-food (Garhwal et al., 2022) and it is used in cosmetics production (Cosentino et al., 2013). There has also been an increasing involvement of donkeys in social activities such as Animal Assisted Interventions (AAI), tourism, and leisure (Amendola et al., 2012; Camillo et al., 2018), changing the social conditions in which they are kept. Thanks to these new socio-economical requests, the latest official report established that 33,116 donkey farms were present on the Italian national territory (30/06/2022; https://www.vetinfo.it/j6_statistiche/#/report-pbi/33). In the traditional use as pack or draught work animals, the domestic donkey population was mainly represented by single individuals or pairs with little or no possibility of interaction with conspecifics (Camillo et al., 2018). However, modern production farms keep donkeys in large social groups which have a variable sex and age composition (Dai et al., 2018). In light of this, a better understanding of the donkey social organization and behavior has become of interest for the owners of donkeys.

To date, the scientific knowledge on the social behavior of domestic donkeys is scarce and mainly restricted to feral populations. A recent review aiming at investigating the scientific literature on donkey behavior and cognition (De Santis et al., 2021) identified the donkey's intraspecific social behavior as the subject of only six scientific articles (excluding those on sexual behavior and on the mother-foal bond). The social organization of feral donkeys is characterized by a marked flexibility on the basis of resource distribution, habitat size, and topography

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(Moehlman, 1998a; Rudman, 1998). This organization of unstable social units and conditional territoriality has been attributed to feral donkeys' ancestry from the African wild ass, which allowed them to successfully exploit new habitats (Rudman, 1998). Under conditions of resource scarcity, feral donkeys can lead lives without long-term social bonds except those between mother and offspring (Moehlman, 1998a; Rudman, 1998). On the other hand, during resource-rich conditions, relatively stable and territorial harem bands of donkeys are found, with the average size of 5 adult animals (McCort, 1980; Moehlman, 1998a, 1998b). High social plasticity enables donkeys to form extremely stable relationships, with long-lasting preferential bonds, which have only been observed in domestic populations (Murray et al., 2013).

The group composition of donkeys does not depend strictly on age and sex (Moehlman, 1998a, 1998b; Rudman, 1998) and dominance hierarchy structures are absent (Proops et al., 2012). Stable groups of feral donkeys can be composed of one or more females and offspring or offspring with variable numbers of females and males (Moehlman, 1998a, 1998b; Rudman, 1998). Furthermore, domestic donkeys form strong pair-bonds with other donkeys, even with unrelated individuals of the same sex (Murray et al., 2013) which, once again, underlines the extreme social plasticity of this species. Therefore, on the one hand domestic donkeys may be extremely easy in adapting to the new social conditions imposed by the current uses for which they are intended. On the other hand, abnormal intraspecific social behaviors of the domestic donkey have already been documented (Brément et al., 2019; Houpt and Antczak, 1998).

The aim of the present study was to deepen our knowledge on the social behaviors of domestic donkeys, which could provide important welfare and management applications for situations where donkeys are housed together. Given the scarcity of data on the social behaviors of domestic donkeys living in captivity, the first goal was to investigate the expression of social behaviors throughout the day. Secondly, we aimed at exploring in detail the expression of agonistic and affiliative behaviors within the group and determine whether patterns of interactions indicative of specific features of domestic donkey's social organization could be identified.

2. Materials and methods

2.1. Subjects and housing

The study took place at an AAI facility located in Northern Italy (Città degli Asini, Polverara (PD), Italy; 45°18.567′ N; 11°57.2844′ E). The AAI activities usually performed in the facility include individual therapies and group activities, which generally consist of approaching, petting, grooming and leading the donkeys.

The study involved thirteen donkeys (10 females, 3 geldings). Among those, six sub-adults (i.e. those ≤ 4 years of age; mean age ± SD = 3.7 ± 0.5 years) had been born at the facility, the remaining seven (mean age ± SD = 9.3 ± 2.2 years) had arrived at the facility, six (N = 5) and four (N = 2) years prior to the study. The animals that had been born at the facility, remained therein since birth (i.e. they were not separated from the group for weaning), thus group composition had been stable since the birth of the youngest donkey, approximately three years before the study. The animals' age, sex and degree of kinship are given in Table 1.

The donkeys lived in a fenced outdoor paddock (Fig. 1). The paddock was divided into two communicating areas (approximately 285 and 195 m^2, respectively), by a wooden fence with a gate, which was usually left open. In the paddock, there were two tree stumps and a few tires, as forms of environmental enrichment. The paddocks were covered with sand. A stable was located in the bigger part of the paddock. The stable was divided into a resting area and an area equipped with two mangers, an *ad libitum* water dispenser, and a salt cylinder. In the smaller part of the paddock there was a large covered square food rack, which the donkeys could access from three sides, allowing for all the donkeys to feed simultaneously.

Table 1
Name, age, sex and degree of kinship of the donkeys (N = 13) involved in the study.

Name	Age (y)	Sex	Degree of kinship
Penelope	8	Female	-
Cleopatra	8	Female	-
Gigliola	3	Female	Michela's daughter, Artù's full sister
Michela	7	Female	Gigliola's and Artù's Mother
Rosa	13	Female	-
Ciuffa	7	Female	May's Mother
May	3	Gelding	Ciuffa's son
Artù	4	Gelding	Michela's son and Gigliola's full brother
Merlino	4	Gelding	Keka's son
Eva	4	Female	-
Ginevra	4	Female	Rossa's daughter
Rossa	10	Female	Ginevra's mother
Keka	10	Female	Merlino's mother

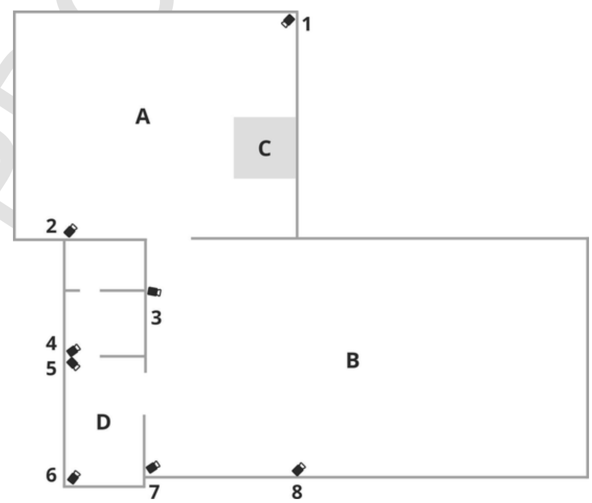

Fig. 1. Schematic aerial view of the donkey's paddock. The two communicating outdoor areas (A and B), the square food rack (C) and the stable (D) are depicted with proportional dimensions. The total dimension of the paddocks was about 480 m^2 and the dimension of the stable was 45 m^2. Numbers 1–8 indicate the position of the cameras used in this study for video-recording.

Cleaning of the pens and feeding took place in the morning between 08:00 and 12:00 AM. Donkeys were fed only hay, which was placed in the food rack and in several smaller piles in the paddocks. AAI involving the donkeys occurred in the afternoons from Monday to Friday, but did not occur regularly. To avoid related effects (Artemiou et al., 2021), the behavioral observations were carried out on days where no AAI had taken place during that day or in the preceding five days.

2.2. Behavioral monitoring system and procedures

For video-recording, we placed eight video-cameras (NetDVR T410, Atlantis Land, Bergamo, Italy): five around the perimeter of the pen and three inside the stable, as detailed in Fig. 1.

The equipment was programmed to record every other hour, beginning at 2:00 PM of a given day and ending at 1:00 PM of the day after. Thus, for each 24 h, we had 12 one-hour videos (*i.e.*, 2:00–3:00 PM; 4:00–5:00 PM and so on).

A total of twenty-seven days between mid-October and mid-December (2017) were recorded. For each day of video-recording, we collected data about meteorological conditions (min - max local temperatures = 3 °C – 17 °C; average relative humidity = 87.5%). Moreover, data regarding donkeys' involvement in AAI or in procedures that were not included in the usual weekly routine (*i.e.*, veterinary therapies, hoof trimming, deworming) during the day of video-recording or the preceding five days was collected. In case the procedure had only involved some of the donkeys, their identity was also recorded.

2.3. Data collection

Before the actual data collection, observers underwent a training for individual recognition of the animals involved in the study, using a photographic database created to highlight differences in physical features. Interobserver reliability for individual recognition of the animals as well as for the behaviors expressed by the donkeys also took place during this preliminary phase using interval scan-sampling recording of a 20 min video of the 13 donkeys. The overall agreement ratio for both was very high, with 1241 scans out of 1288 (96.4%).

Data collection was carried out in two successive phases. In the first phase, the data collection aimed at exploring the circadian distribution of donkeys' intraspecific social behavior (henceforth referred to simply as social behavior, if not stated otherwise) and at identifying the period of the day in which most of the social behaviors took place. In the second phase, the data collection aimed at characterizing in greater detail affiliative and agonistic behaviors (*e.g.*, the type of behaviors, their frequency and mean duration, and the identity of donkeys involved). For this second phase, observations were focused on the period of peak activity, as resulting from analysis of the previous phase. Data collection in the two phases was performed using different sampling methods and ethograms, as detailed below. The working ethograms were developed based on preliminary direct *ad libitum* behavioral observations and the existing literature on the topic (McDonnell, 2003; Moehlman, 1998b). Vocalizations were not collected, because the video cameras did not record sound.

For the first phase, we selected three non-consecutive days, for a total of 36 hours, among those in which recording occurred. We selected days in which there was no heavy rain and in which no AAI or extraordinary interventions had taken place during that day or in the preceding 5 days. We used an instantaneous focal/scan animal sampling and recording method (Bateson and Martin, 2021) every 20 seconds and analyzed the first 20 minutes of each one-hour video for a total of 2196 scans per donkey. For each scan, the name of the donkey expressing the behavior and the category of behavior expressed were collected. The mutually exclusive behavioral categories we recorded in this phase were: affiliative behavior towards other donkeys, agonistic behavior towards other donkeys, other social behavior (*e.g.,* sexual behaviors, affiliative or agonistic behaviors towards people), non-social behavior (*e.g.*, resting, inactivity, locomotion, body-care, feeding, drinking, interactions with the inanimate environment, other non-social behavior) and out of sight (the observer could not see the donkey or could not identify the behavior it was expressing).

In the second phase, we selected four days among the recorded ones, in which there was no heavy rain, no AAI and no extraordinary interventions had taken place during that day or in the preceding 5 days. We used a continuous behavioral sampling and recording method (Bateson and Martin, 2021) to collect data from the hour of peak social activity as resulting from the first phase data (*i.e.*, from 8:00–9:00 AM). For each individual donkey we collected all occurrences and duration of diverse types of affiliative and agonistic behaviors (as defined in Table 2).

Table 2
Working ethogram used to characterize interspecific affiliative and agonistic behaviors expressed by donkeys.

Affiliative Behaviors	
Proximity	A donkey is maintaining proximity (< 1 m) to one or more donkeys while stationary and not performing any other behavior (King and Gurnell, 2019). Interactions at the feeding rack were excluded.
Sniffing / Mutual sniffing	A donkey repeatedly inhales and exhales with its muzzle positioned near any part of the body of another donkey (McDonnell, 2003).
Allogrooming	Two donkeys standing, parallel and facing opposite directions, and in unison scraping the hide of the other with its upper incisors (Moehlman, 1998b).
Follow	A donkey moves along, behind another donkey (< 1 m distance) usually at the same gait, without being forced by circumstances to do so, neither attempting to direct the movement of the other donkey, attack it (no threats, such as lowered ears) or overtake it (McDonnell, 2003).
Contact	A donkey puts a part of its body in contact with a part of the body of a conspecific (usually the head on the shoulder or on the croup).
Nursing and Suckling	Contact between the mother donkey's udder and the offspring's mouth.
Flehmen	A donkey has the head elevated and neck extended, eyes rolled back, ears rotated to the side, and upper lip everted to expose the upper incisors and gums, while drawing air and fluids through the mouth. Often associated with olfactory investigation (McDonnell, 2003).
Agonistic Behaviors	
Avoidance	A donkey maintains or increases the distance to another approaching or threatening donkey, before contact between the two is established (McDonnell, 2003).
Displacement	A donkey causes a conspecific to move away by means of applying pressure with a part of its body against the body of the conspecific without kicking or biting (Giles et al., 2015).
Threat	A donkey shows mimic and postural signs, which convey its readiness to engage in an agonistic confrontation. The signs can consist in positions of the ears (often flattened against the neck), head carriage, legs, and total body positioning. Frontal threats can vary from a fast head movement (with or without flattened ears) toward another donkey, to approaching at different gaits with neck extended and lowered head, to a bite threat with open mouth teeth exposed and flattened ears. Rear threats included 'pivot rump' toward another donkey (with or without flattened ears), backing toward the approaching or standing receiver, lifting one rear hoof inches off the ground, drawing the rear hoof under the belly in preparation for a backward kick, and one or two-legged kick into the air. During threats there is no contact with the individual to whom the threat is directed (Moehlman, 1998b).
Kick	A donkey lifts one or both hind legs off the ground, and rapidly extends backward towards another donkey with apparent intent of making contact (McDonnell, 2003).
Bite	A donkey is rapidly opening and closing the jaws with the teeth grasping the skin of another donkey. Lips are retracted and ears pinned (McDonnell, 2003).
Chase	A donkey approaches another donkey, at any gait, but usually at a canter, in an apparent attempt to overtake, direct the movement of, or catch up with the other individual. The pursuing individual often shows lowered head, pinned ears and exposed teeth (McDonnell, 2003).
Driving / Herding	A donkey expresses a combination of forward locomotion with ears pinned, extended and lowered neck and head in an apparent attempt to direct the movements of another donkey (McDonnell, 2003). Teeth are not exposed in a bite threat as usually during "Chase" and the animal does not appear to attempt to reach the other individual.

2.4. Statistical analysis

Data from the first phase was analyzed using Generalized Estimating Equations (GEE) models (Hardin and Hilbe, 2002) to explore the distribution of behavior across the day. However, a high proportion of out of sight for all donkeys was observed in the intervals between 6 PM and 7 AM, for it resulted impossible to reliably recognize the donkeys on images obtained in the dark. Therefore, data obtained in these time intervals was excluded from the analysis. As regards the remaining intervals, we excluded from the analysis the data of individual donkeys, if the donkey was out of sight for ≥ 50% of the interval.

In a first GEE model, the number of scans in which donkeys were observed performing either affiliative or agonistic behaviors were summed to obtain a single variable, divided by the total number of scans in which the donkeys were visible (hereafter: frequency of the social behaviors) which was used as dependent variable in the model. The specified distribution was normal with identity link. The time of the day was included as fixed factors and the donkey's name as random factor to account for repeated data collected from the same animals. Post-hoc comparisons with sequential-Bonferroni corrections were performed when time of the day resulted significant.

Other four separate GEE models were run for the proportion of scans in which the donkeys were observed expressing respectively affiliative, agonistic, non-social behaviors or when they were out of sight. The specified distribution was normal with identity link. The time of the day, the age class (adults, sub-adults) and their interaction were included as fixed factors and the donkey's name as random factor to account for repeated data collected from the same animals. Post-hoc comparisons were performed when factors or the interaction resulted significant. Sequential-Bonferroni correction was applied to comparisons. In all cases, test of model effects was used as outcomes.

Data collected in the second phase was used to calculate frequency and duration of the social behaviors (Table 2). The data was divided into affiliative interactions and agonistic interactions and organized into two separate matrixes. The affiliative interactions matrix indicated the number of affiliative interactions between any given pair of donkeys. The agonistic interactions matrix indicated the number of agonistic interactions between any given pair of donkeys, as well as which of the two donkeys initiated the behavior (initiator) and to whom the behavior was directed (receiver). In case of avoidance, the donkey causing the other to increase the distances was considered the initiator.

Based on the matrixes, two weighted social networks were built. Nodes in the networks represented individual donkeys and edges represented their interactions, where the edge weight was based to the number of observed interactions. The network based on the affiliative interactions (hereafter: affiliative network) was undirectional. The network based on the agonistic interactions (hereafter: agonistic network) was directional *i.e.,* arrowed edges were outgoing from the initiator and incoming to the receiver. For both networks we calculated the modularity index (Q), which measures the proportion of edges that occur within sub-communities of the network, relative to the expected proportion if all edges were placed randomly across the network (Newman, 2006). Moreover, for each node a weighted degree centrality was calculated for both networks. For agonistic network, also the weighted in- and outdegree centralities were calculated.

To assess if the expression of affiliative and agonistic behaviors differed between adult and sub-adults, the respective weighted degree centralities were compared between the two groups, using a two-samples permutation test. For agonistic interactions in- and outdegree centralities were also compared. The same test was used to assess if the expression of social behavior differed between donkeys identified by the affiliative network as belonging to pairs compared to the ones belonging to a group of three or more.

The steepness of the dominance hierarchy was assessed by calculating normalized David's scores based on agonistic interactions, plotting the resulting data against the rank of the donkeys, from highest to lowest and calculating the curve's slope, as described by De Vries et al. (2006).

The social network analysis was computed using Gephi (Bastian et al., 2009) and all other statistical analysis were done with SPSS (ver. 27, IBM, Armonk, NY, USA).

2.5. Ethical approval

The study was conducted in accordance with relevant legislation for research involving animals, and according to the type of procedure used, no formal ethical approval was required.

3. Results

3.1. Circadian distribution of donkeys' social behavior

On a total of 28,548 scans, donkeys were out of sight for 14,465 scans (50.7%), they were not involved in any social behavior for 11,901 scans (41.7%) and were involved in social behavior for 2182 scans (7.6%). Donkeys were thus involved in social behavior in 15.5% of the scans in which they were visible. Among social behavior, we recorded affiliative behavior towards another donkey in 1957 scans (6.9% on the total), agonistic behavior towards another donkey in 202 scans (0.7% on the total) and other social behaviors in 23 scans (<0.1% on the total; sexual behavior = 18, affiliative behavior towards humans = 5).

The GEE on the frequency of social behaviors expressed by donkeys revealed a significant effect of the time of the day (Wald Chi-square = 28.7, $P < 0.001$). Most of the social interactions were observed between 8:00 – 9:00 AM (corrected $P < 0.05$ for comparisons with all other intervals), while no difference was found amongst other intervals.

Affiliative behaviors were affected by both the time of day (Wald Chi-square = 25.3, $P < 0.001$) and the age-class of the donkey (Wald Chi-square = 4.1, $P = 0.042$). Specifically, they were more often observed in adults than sub-adults and were more often observed between 8:00 – 9:00 AM than all other intervals (corrected $P < 0.05$ for all comparisons), except 2:00 – 3:00 PM (Fig. 2). For agonistic behaviors a significant effect of the interaction between the age class and time of day was found (Wald Chi-square = 26.3, $P < 0.001$). Specifically, agonistic behaviors were expressed less by adults between 2:00 – 3:00 PM than 8:00 – 9:00 AM and 4:00 – 5:00 PM (corrected $P < 0.05$), whereas no difference was found between time intervals for sub-adults (Fig. 2). Non-social behaviors were affected by both the time of day (Wald Chi-square = 24.1, $P < 0.001$) and the source of the donkey (Wald Chi-square = 4.1, $P < 0.042$). Specifically, these behaviors were less expressed adults than sub-adults and were less expressed between 8:00 – 9:00 AM than any other time interval (corrected $P < 0.05$ for all comparisons) (Fig. 2).

Considering the intervals between 8:00 AM and 5:00 PM, affiliative behaviors were expressed for 19.1 ± 19.1% (mean ± SD) of the time in which the donkeys were visible, implying a coefficient of variation of 1. Details about the hourly distribution of affiliative behavior expressed by individual donkeys are reported in Table S1.

3.2. Affiliative and agonistic interactions during peak hours

A cumulative total of 290 social behavior occurrences were observed across all the study days in the time frame between 08:00 – 09:00 AM. More specifically, 188 were affiliative behaviors (frequency: 47.0/h) and 102 were agonistic behaviors (frequency: 25.5/h). Table 3 details number of occurrences and mean duration of the recorded social behavior.

Among affiliative behavior, the most expressed one was proximity (55.0%) and the least expressed ones were flehmen and nursing/suckling which were only observed once (0.5%). Fig. 3 shows the affiliative network. The modularity index of the network was 0.55,

indicating that the network could be divided in clusters *i.e.*, that more frequent interactions were found between animals belonging to the same cluster than between other animals (Newman, 2006). Five clusters were identified: three dyads, one triplet and one quartet (Fig. 3). Two of the dyads were composed of a mother and an offspring (Ciuffa and May; Michela and Gigliola), while the other dyad was composed of two adult females. The cluster of three individuals included only adults, whereas the cluster of four animals included only sub-adults. The average weighted degree centrality of the affiliative network was 33.1 ± 18.8; individual values for each subject are shown in Table 4.

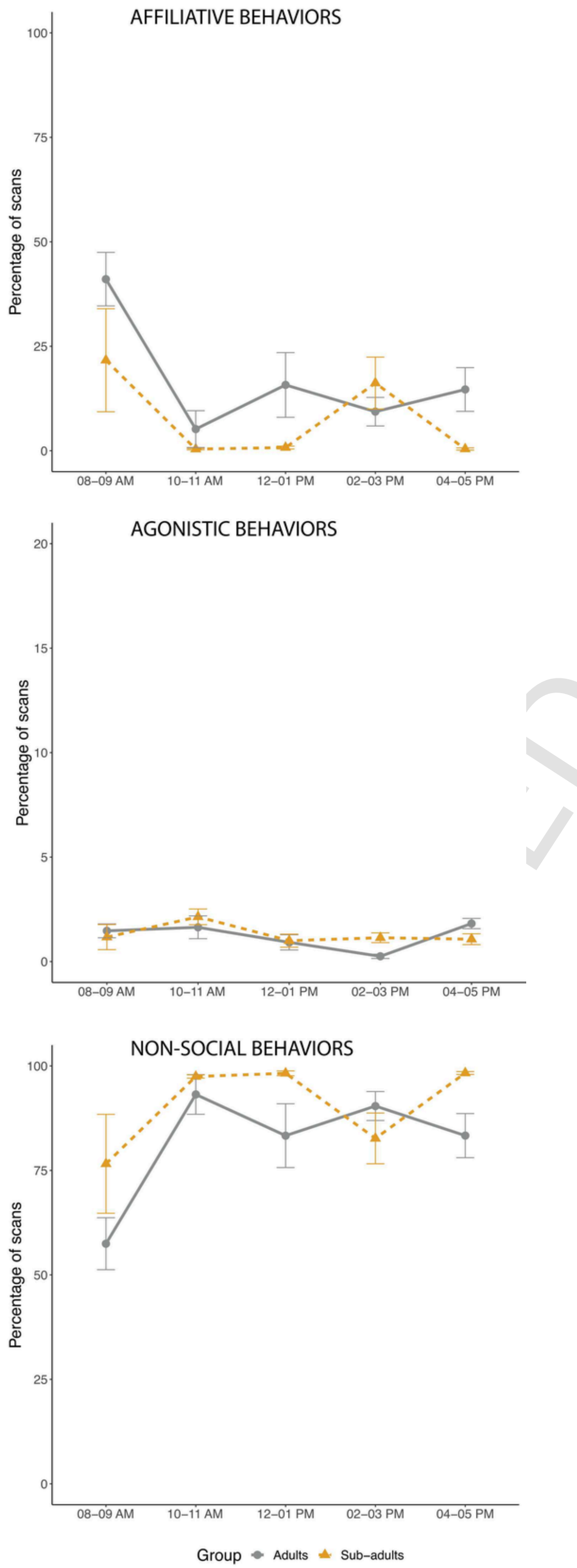

Fig. 2. Distribution of affiliative, agonistic and non-social behaviors by adult (ochre dashed line) and sub-adult donkeys (grey line).

Table 3
Total number of occurrences and mean duration of affiliative and agonistic behaviors expressed by donkeys (N = 13) in the hour of most frequent social interaction (08:00 – 09:00 AM) across four days, for a total of four hours of observation.

Affiliative behavior	Number of occurrences	Mean duration (s)
Proximity	104	212.0
Follow	55	15.9
Sniffing	21	4.8
Allo-Grooming	4	6.0
Contact	2	17.5
Flehmen	1	4.0
Nursing/suckling	1	51.0
Agonistic behavior	**Number of occurrences**	**Mean duration (s)**
Threat	52	3.4
Bite	19	2.4
Displacement	19	4.5
Avoidance	7	3.6
Chase	4	7.3
Kick	1	2.0
Herding	0	-

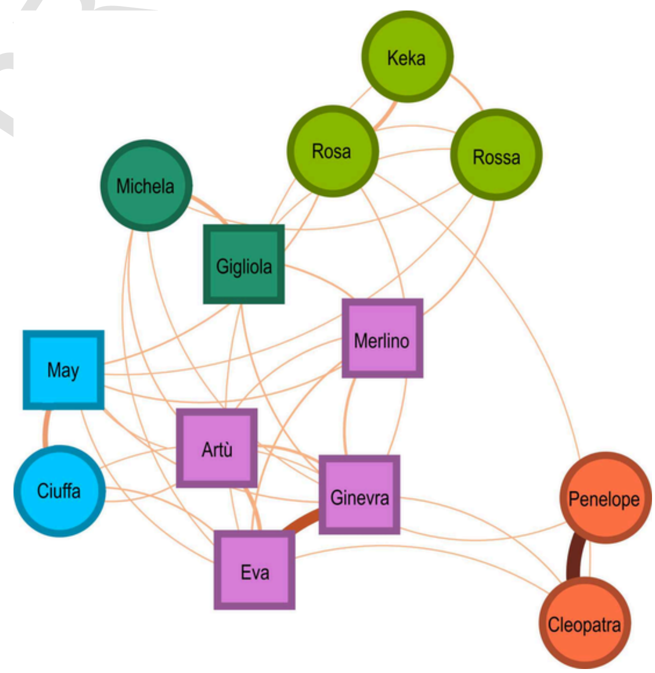

Fig. 3. Weighted unidirectional social network, based on affiliative interactions during the hour of peak social activity, over four days. Colors identify nodes belonging to modularity classes. The shape of the node indicates whether the animal was introduced (circles) or born in the group (rectangle). The thickness and darkness of lines between nodes is proportional to the number of interactions between the two donkeys.

The most expressed agonistic behavior was threat (51%), followed by displacement and bite (both 18.5%), avoidance (7%), chase (4%) and kick (1%). Fig. 4 shows the agonistic network. The modularity index was 0.25, indicating that the network could not be divided in clusters based on agonistic interactions (Newman, 2006). The weighted degree centrality of the agonistic network was 14.8 ± 7.3, and the weighted in- and outdegree centralities were 7.4 ± 4.3 and 7.4 ± 4.2, respectively. Individual values for each subject are reported in Table 4.

Permutation tests revealed no difference in the weighted degree centralities of the either the affiliative or the agonistic network between the adult and sub-adult donkeys. Donkeys clustered in groups based on the affiliative network had a higher weighted degree centrality of the agonistic network than those clustered as pairs (mean ± SD (groups) = 18.7 ± 7.5, mean ± SD (pairs) = 10.2 ± 3.8; P = 0.029). No difference was found between such two groups in the weighted degree cen-

Table 4
Individual values of the weighted degree centrality for the affiliative and weighted degree, indegree and outdegree centrality for the agonistic network.

Affiliative network		Agonistic network		
Subject	Weighted degree centrality	Weighted degree centrality	Weighted indegree centrality	Weighted outdegree centrality
Penelope	56	13	8	5
Cleopatra	58	11	7	4
Gigliola	21	3	1	2
Michela	18	9	6	3
Rosa	20	21	10	11
Ciuffa	24	12	4	8
May	28	13	5	8
Artù	27	20	13	7
Merlino	21	15	6	9
Eva	61	4	3	1
Ginevra	63	24	12	12
Rossa	13	20	5	15
Keka	20	27	16	11

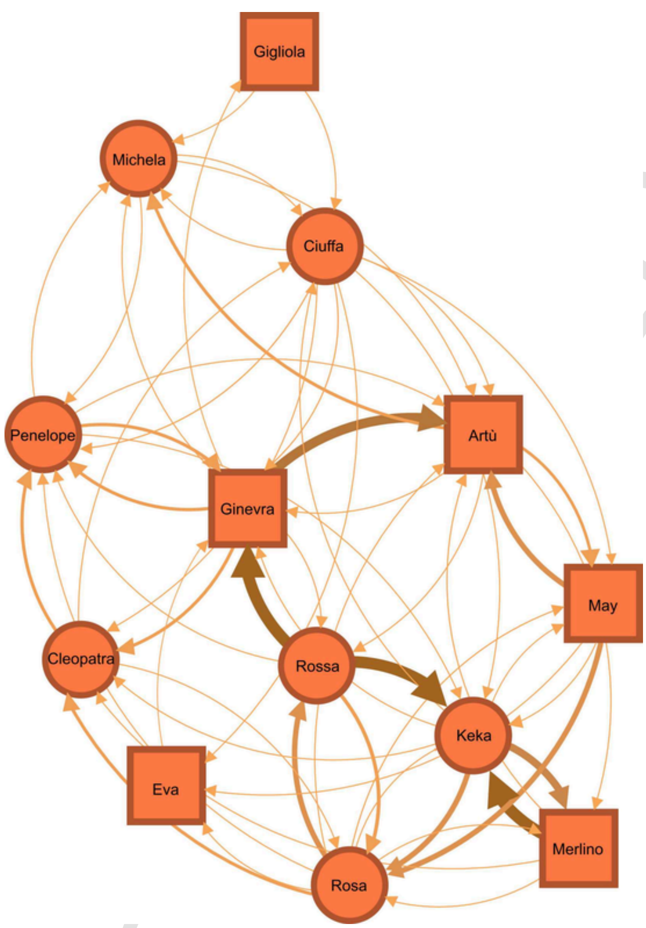

Fig. 4. Weighted directed social network, based on agonistic interactions during the hour of peak social activity, over four days. The shape of the node indicates whether the animal was introduced (circles) or born in the group (rectangle). The thickness and darkness of lines between nodes is proportional to the number of interactions between the two donkeys. Arrows at the end of lines point to the direction of the receiver of the agonistic behavior.

trality of the affiliative network, or in the weighted indegree centrality and the weighted outdegree centrality of the agonistic network.

The mean ± sd normalized David's score was 5.9 ± 03, resulting in a curve with a slope of 0.08, indicating the substantial absence of a linear hierarchy.

4. Discussion

Most of the social behaviors observed within the herd were affiliative, and proximity to another donkey constituted the largest part of these. Affiliative behaviors were slightly more often expressed at the onset of day than in most of the rest of daytime. Since domestic donkeys are a gregarious (Murray et al., 2013) and diurnal species (Lamoot et al., 2005; Zakari et al., 2018), it is possible that a high morning expression of affiliative behavior serves to re-initiate social interactions after the nightly rest. It is also possible that affiliative social interactions decreased after 9:00 AM for being replaced by foraging. Food was generally provided after cleaning, which started around 8 AM, and foraging would have therefore competed with affiliative behaviors on subsequent intervals; in fact, non-social behaviors, of which foraging was part, were affected by time in a specular way compared to affiliative behavior. It must also be considered that this slight flexion in affiliative behavior might be only apparent: affiliative behaviors were mainly constituted by proximity, which donkeys might have maintained during feeding, although not captured by our data collection, as the two behaviors were mutually exclusive.

Affiliative behaviors were expressed more often by adults than sub-adults. However, the difference does not seem due to a generalized age-related increase in affiliative behavior,

which would also disagree with existing literature in other equids (Pierard et al., 2019), but mainly to a low expression of affiliative behavior by the oldest amongst the sub-adult. In turn, this was associated with different social preferences by these animals. Indeed, donkeys who expressed more affiliative behaviors, did so towards another specific individual, as evident by the identical number of interactions observed within pairs. This was the case of most of the adults and of the two youngest donkeys; conversely, no preferential association was observed in the oldest sub-adults, who were also generally expressing rarer affiliative behaviors.

The dyads involving the youngest donkeys were composed of a mother and her offspring. These close preferential spatial associations are unsurprising: mother-foal dyads are the most common cohesive and persistent forms of association in feral asses, especially under conditions of resource scarcity (Moehlman et al., 1998a). Although the spatial patterns of proximity between jenny and offspring change considerably in the first year of age, close association may persist well after weaning (French, 1998 and references therein). In semi-feral horses, bonds between a mother and a sub-adult offspring have been suggested to act as a mechanism delaying the dispersal of young animals and increase their survival rate (Stanley and Shultz, 2012). The limited expression of affiliative behavior by the older sub-adults in our group, is in accordance with the notion such preferential association can be disrupted after three years of age. This corresponds with the time donkeys complete their growth (Choquenot, 1991) and leave their mothers to join groups of bachelors (McCort, 1980). In wild asses, interactions by young adults occur preferentially with age-matched individuals (Kozlowski et al., 2021), rather than other non-maternal adults. In accordance, the four sub-adult donkeys in our study were identified as part of the same sub-group by the social network analysis, which also implied that they did not have a single preferential partner.

Preferential dyadic affiliative interactions were observed between adults not involved in a mother-foal association, and again associated with high frequency of expression of such behavior. The result highlights the relevance of this form of association for domestic donkeys, converging on this as a predominant feature of the species' social organization, as previously suggested (Murray et al., 2013; Wasilewski, 2003). The adult donkeys had all been introduced in the social group from outside. Since age class and source were confounded, it is impossible to determine how the two factors contributed to the results and both have been proposed to play a role in the formation of bonds (Murray et al., 2013). However, the fact that these animals still interacted in preferential pairs, highlights donkeys' adaptability upon changes in the social context. Again, this is in line with reports by Murray and collaborators (2013) showing that pair-bonds are unaffected by changes in group composition.

In summary, results about the characterization of affiliative interactions suggest that donkeys tend to engage in associations with preferred partners, with whom they maintain spatial proximity. For younger animals the preferred partner is represented by the mother. The tendency seems to transitorily decrease, potentially accompanied by an overall reduction in expression of affiliative behavior, when donkeys reach full maturity and engage in less exclusive interactions with other conspecifics, before eventually becoming again apparent in adulthood.

Agonistic interactions among the donkeys were much rarer than affiliative ones. This is in sharp contrast with the behavior of Somalian wild asses, where aggressive interactions were found to be more common than affiliative behaviors (Asa et al., 2012; Marshall and Asa, 2013), which might reflect the species' tendency towards a solitary social organization and territoriality. Although the domestic donkey is believed to be derived from other, now extinct, populations of African asses, for which we do not have details about the social organization, it is sensible to believe that domestication relaxed the pressure on the competitive advantages of a solitary life, and increased tolerance and gregariousness. Indeed, domestication in many cases reduces aggressive behavior and increases sociality (Jensen, 2014). The availability of sufficient resources, granted by the domestic environment, might also have contributed to a low expression of agonistic behavior. In fact, resource availability is an important contributing factor on the formation of relatively large, stable social units in feral donkeys (Moehlman et al., 1998a). It must be noted, however, that agonistic interactions were less often expressed by donkeys who had a preferential partner, based on the affiliative network, than those who belonged to groups. This suggests that reduction in agonistic behavior is associated with an actual change in social preferences, not a mere consequence of fewer occasions of conflict over resources. However, the direction of the causality *i.e.,* whether having a preferential association reduces the need or the opportunities to engage in agonistic interactions, or rather if the low level of agonistic interactions allows donkeys to form preferential associations, remains an open question.

In line with the previous literature (Proops et al., 2012) there was no indication of a linear hierarchy, as indicated by the almost flat regression of normalized David's scores. Accordingly, almost half of the observed agonistic interactions were overt aggression, rather than threat; conversely, the ratio between aggression and threat is much lower in mules and horses, which are characterized by a clearer linear hierarchy (Narciso et al., 2021). The lack of a linear hierarchy in domestic donkeys could reflect the predominantly solitary social organization of the wild ancestor(s), which implies a limited selective pressure to develop a hierarchical system for negotiating competitions without resorting to overt fights.

This study has some relevant limitations. First, we only observed a single herd, composed by a relatively small number of animals. Therefore, the behavior of animals in our sample cannot be considered independent of that of the other animals in the group, nor the sample representative of the donkey population. Yet, in view of the scarcity of data about the social behavior of domestic donkeys, the result here reported represent a useful addition to the existing literature and will hopefully represent a starting point for further research. Another aspect that should be considered, is that the source of the donkeys – whether they were born in the facility or introduced from outside – co-varied with the donkeys' age. Therefore, the effects of source and age could not be disentangled, and further experiments would be needed to isolate the contribution of these two factors on donkey's social behavior. Finally, our sample did not include enough males and females to analyze potential contribution of sex to the expression of social behavior; however, sex is a potentially important factor in affecting social preferences in donkeys (Moehlman et al., 1998b; Rudman, 1998). Again, this factor will need to be addressed by future studies.

5. Conclusions

The current study provides some insights into the intraspecific social interactions of domestic donkeys. Donkeys' social behavior is characterized mainly by affiliative interactions, which are far more common than agonistic ones. Affiliative behaviors are commonly expressed within consistent pairs, providing support to the idea, suggested but not extensively proven by other research, that this is a predominant feature of the social organization of the species. This feature differentiates domestic donkeys by most feral and wild asses populations, where aggregations are rarer and dyadic associations often limited to mother-offspring pairs.

Certainly, the current study is limited in breadth for a limited sample size and observation of a single herd. For this, future research should aim to extend these results on larger samples and multiple groups. Also, studies should aim at providing a better understanding of conditions which allow the formation of pair bonds, as well as of the mechanisms linking such preferences with the expression of agonistic behaviors. Several factors may be at play in determining social preferences, potentially including sex, age, provenance, previous history, and pre-existing relationships with other animals in the group. In addition, resource distribution and availability might be another factor affecting the expression of affiliative and agonistic behavior and the formation of larger social units. All of these are potential aspects that will have to be addressed in the future. This seems to be especially relevant in view of the increasing popularity of donkeys' farming, and of the potential impact of a correct management of these animal's social context on their welfare.

CRediT authorship contribution statement

Each author declares substantial contributions through the following:

(1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content,

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2023.106132.

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