Social determinants of affiliation and cohesion in a population of feral horses

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Abstract

Investigating affiliative behaviors (e.g. proximity, grooming, cooperative behaviors) is essential to understand group cohesion and stability in animal societies, as they may foster, or be fostered by long-term social bonds and ultimately determine an individual's lifetime reproductive success. Despite growing interest in affiliative behaviors across a wide range of mammalian taxa, little focus has been given to feral horse populations. In this study, we examined the influence of dyadic social factors (sex, rank difference, familiarity and genetic relatedness) on affiliative behaviors and group cohesion. We collected data on 16 feral horse groups in Northern Portugal who were subjected to both predation pressures and anthropogenic interference. A combination of all occurrence sampling methods and drone technology was used to record the horses' social behavior and spatial data. Same-sex dyads showed a preference to remain in close proximity, however they did not groom as frequently as different-sex dyads. Similar rank individuals were observed in proximity more

often than non-similar rank individuals, but only in newly formed groups. Familiar individuals spent more time in proximity and groomed more often than unfamiliar (new) dyads. However, affiliation between conspecifics was not predicted by genetic relatedness. These results suggest that social bonds and group cohesion are mostly sustained by familiar individuals in both stable and newly formed groups. While maintaining close proximity may function to sustain bonds between same-sex individuals, grooming may reinforce both intra-sexual and inter-sexual bonds. Examining the variation of affiliation patterns on horse populations may shed light on their behavioral plasticity and have crucial implications for the welfare and conservation of horse breeds.

1. Introduction

Group living provides numerous benefits for social animals including reducing predation risk (Alexander, 1974, Krause and Ruxton, 2002), improving foraging strategies (Galef and Giraldeau, 2001, Giraldeau et al., 1994) and facilitating information transfer via social learning (Krause et al., 2010, Ashton et al., 2019). Animals maintain group cohesion by making collective consensus decisions (Conradt and Roper, 2000) and/or adjusting inter-individual distance (Krause and Ruxton, 2002). Affiliation among group members may affect group cohesion as well by increasing spatial proximity (i.e., smaller distances) between group members (Cords, 2002, Sussman et al., 2005, Lehmann et al., 2007). Hence, affiliation can be either regarded as a source or as an outcome of long-term bonds among conspecifics; it can be expressed in a variety of ways, including spatial proximity (Gero et al., 2008, Wolf and Trillmich, 2008, Cameron et al., 2009, Dolotovskaya et al., 2020), physical contact (e.g., grooming: Silk et al., 2006; Perry et al., 2008, Jablonski, 2020), play behaviors (Sharpe, 2005), formation of alliances and coalitionary support (Smith et al., 2010, Gilby et al., 2012) and allomaternal care (O'Brien and Robinson, 1991, Jesseau et al., 2009). Homophily, also known as the tendency to interact with individuals sharing similar traits (McPherson et al., 2001), has been described in a wide variety of animal taxa for several traits: sex (Smith et al., 2002, Ruckstuhl, 2007), age (Smith et al., 2002, Silk et al., 2006, Wolf et al., 2007, Wey and Blumstein,

2010), kinship (Silk, 2002, Wey and Blumstein, 2010, Wiszniewski et al., 2010), familiarity (van Dierendonck et al., 2004, Pinter–Wollman et al., 2009), reproductive status (Sundaresan et al., 2007, Möller and Harcourt, 2008), behavioral phenotype (Pike et al., 2008), phenotypic traits (reviewed by Krause et al., 2000) and even temporary physiological states such as hunger (Romey and Galbraith, 2008). Homophily has been predominantly studied on species with complex social structures, such as fission-fusion or multilevel societies. Hence, feral equids seem to be ideal subjects for exploring homophily due to the characteristics of their social system and social bonds (Bouskila et al., 2016; Maeda et al., 2021). Feral horses establish year-round, socially stable and bisexual breeding groups, called harem groups, that consist mainly of one male (although two or more male groups have been widely observed) and several unrelated females with their immature offspring (Berger, 1986, Rubenstein, 1994, Stanley et al., 2018). They also form all-male groups, known as bachelor groups (Linklater, 2000). Before the onset of adulthood, both young male and female horses disperse from their natal groups. This process of dispersal is suggested to have evolved to avoid incest (Linklater and Cameron, 2009, Monard et al., 1996). Thus, horses must develop bonds with newly acquainted and unrelated conspecifics in order to integrate into a new group (Monard and Duncan, 1996). Horses' strong and long-term inter-sexual social bonds are proposed to have evolved as a strategy to reduce intra-group and outgroup harassment, as well as to promote group stability and cohesion to cope with predation pressures (Feh, 1999, Linklater et al., 1999, Cameron et al., 2009). Furthermore, intra-sexual bonds between unrelated females have been reported to increase females' reproductive success by increasing birth and offspring survival rates (Cameron et al., 2009). Similar to many other species that form long-term stable bonds (hyenas, Crocuta crocuta: Smith et al., 2007; elephants, Loxodonta africana: Archie et al., 2006; primate species: Silk, 2007), horses form clear dominance relationships among group members which are mostly linear and relatively stable over time (e.g., Sigurjónsdóttir et al., 2003; van Dierendonck et al., 2004; Heitor et al., 2006a). Investigating horses' affiliation preferences and the social factors underlying the formation of their

social bonds is crucial to understand how their relationships are established and maintained over time.

Previous studies have reported that horses tend to form bonds with individuals sharing similar traits and that these preferences could reflect similar social and ecological needs (sex and age class: Clutton-Brock et al., 1976; Sigurjónsdóttir et al., 2003, reproductive stage: van Dierendonck et al., 2004; Heitor and Vicente, 2010; Bouskila et al., 2016 and rank: Ellard and Crowell-Davis, 1989; Kimura, 1998; Sigurjónsdóttir et al., 2003; but see: van Dierendonck et al., 2004). However, the effect of familiarity on affiliation is poorly understood as most studies focus on groups or herds consisting of already familiar individuals (but see: Monard et al., 1996; van Dierendonck et al., 2004). The role of genetic relatedness in horses deserves further investigation as well. Despite some studies reporting a preference to affiliate with kin (Gilbert-Norton et al., 2004, van Dierendonck et al., 2004, Heitor et al., 2006b), others have failed to show a positive relationship between them (Clutton-Brock et al., 1976; van Dierendonck et al., 1995; Heitor and Vicente, 2010; Bouskila et al., 2016). According to Hamilton's kin selection theory, which predicts that individuals can gain indirect fitness benefits by cooperating with kin (Hamilton, 1964), we would expect horses to show a preference towards affiliating with closely related conspecifics. Therefore, due to the horses' natal dispersal pattern the likelihood of close kin affiliations may be low. Other theories may offer better explanations for the evolution of cooperation and long-term bonds between non-kin individuals (see for a review: Nowak, 2006); as for example Triver's theory of the Prisioner's Dilemma, based on direct reciprocity (Trivers, 1971).

Most former studies targeted a single group or herd of horses subjected to different management policies and used varying methods for data collection and analyses, hindering cross-studies comparisons and thus the ability to draw precise conclusions (for a review, see Costa et al., 2019). The present study attempted to address these concerns using a standardized and replicable methodology to examine the affiliative behaviors of feral horses at a population level, while acknowledging separate horse groups with different social compositions. The effect of different social factors (sex, rank difference, genetic

relatedness and familiarity) on affiliation (proximity and mutual grooming) and group cohesion (inter-individual distance) was examined by combining behavioral observations and drone technology. This allowed us to measure inter-individual distance simultaneously and more accurately (Inoue et al., 2018, Inoue et al., 2020, Ringhofer et al., 2020, Maeda et al., 2021). Considering the social system and dispersal patterns of horses, we predicted that 1) horses would show a preference towards affiliating with group members of the same-sex, similar rank and with familiar individuals, 2) genetic relatedness would have a weak effect on affiliation between conspecifics and 3) sex, rank difference, as well as familiarity, would be the main factors determining group cohesion. Furthermore, we investigated affiliation in groups with different levels of stability and discussed the potential functions of two affiliative behaviors, proximity and mutual grooming.

2. Material and methods

2.1. Study site and population

The study was conducted in Serra d'Arga (825 m a.s.l.), a mountain range comprising an area of 4493 ha in northern Portugal (8° 42′ N, 41° 48′ E). This region is characterized by a Mediterranean climate with Atlantic influence, a mean annual precipitation over 2800 mm and a mean annual temperature of 12.2 ° C (Pereira et al., 2008; Gonçalves et al., 2016). The high altitude plateau (700–800 m a.s.l.) consists mainly of wet and shrub lands dominated by heather species (Erica ciliaris, Erica tetralix, Calluna vulgaris), gorse (Ulex minor, Ulex europaeus) and Genista species (G. anglica, G. micrantha) interspersed with extensive granite outcrops. The lower altitude landscape (around 300–400 m a.s.l.) is comprised mostly of agricultural fields, pine forests (Pinus pinaster) and eucalyptus forests (Eucaliptus globulus) (Pereira et al., 2008, Gonçalves et al., 2016). During the breeding season (May to August), the high-altitude plateaus are home to a breed of horses endemic to Portugal and Galicia, Spain, named Garrano (Morais et al., 2005). The feral horse population inhabiting this area is privately owned by livestock farmers, however horses are not managed under the traditional husbandry system

observed in other populations in Galicia and Northern Portugal where yearly roundups occur to capture foals to be sold for meat (Lagos, 2013, Nuñez et al., 2016). The removal of Garrano horses in Serra d'Arga takes place arbitrarily, although it usually occurs after the breeding season and the majority of individuals taken away are male. Moreover, the Garrano population in Serra d'Arga is subjected to predation pressures by the Iberian wolf (Canis lupus signatus; Álvares, 2011) which has been the main cause of population decline over the last four years, alongside inadequate management practices (Freitas, 2019). Field research on this population has been ongoing since 2016; researchers have mainly focused on the horses' social system and spatial-social behaviors using novel technologies and non-invasive methodologies (Ringhofer et al., 2017, Ringhofer et al., 2020, Inoue et al., 2018, Inoue et al., 2020, Mendonça et al., 2020, Pinto and Hirata, 2020, Maeda et al., 2021).
So far, 35 harem groups and around 200 individuals have been identified. In the 2018 breeding season, the Serra d'Arga population consisted of 25 harem groups (20 of which were one-male groups and five two-male groups) ranging from two to nine individuals (group size of 5.40 $\pm$ 1.88 individuals, mean $\pm$ SD), as well as 13 bachelor males. From August 2018, the population suffered a severe decline resulting in a 2/3 decrease in size. Consequently, changes in group compositions were observed. In the 2019 breeding season, the population comprised 11 harem groups: nine one-male groups and two two-male groups ranging from two to six individuals (group size of 4.54 $\pm$ 1.16 individuals, mean $\pm$ SD) and no bachelor males. The sex ratio during the study period varied from 2.15 to 2.62 females per male in 2018 and 2019, respectively. All individuals were given names and identified based on visible phenotypic traits such as body color, presence/absence and shape of white marks on the face and/or feet as well as the color and direction of mane. Groups were identified by the name of the male in the group, or in the case of two-male groups, both of their names.

2.2. Data collection

2.2.1. Behavioral data

Data was collected between 9:30 and 18:00 on a daily basis during the 2018 and 2019 breeding seasons (May to August in 2018 and May to July in 2019) for a total of 205 h on 16 horse groups (Table 1). In both years, groups were observed for 1 h per day (never more than once per day) and observations were distributed evenly across the groups and hours of the day. One to six groups were followed each day depending on weather conditions and group dispersion. Fog and rain occasionally prevented the collection of behavioral observations due to the limitations of the equipment used. Observations were made on foot and horses could be observed from a distance of 5–10 m without showing signs of disturbance. A minimum of two researchers were necessary to identify and follow a single group; while one person recorded the horses using a video camera, the other person operated the drone. Additionally, volunteers assisted the researchers. In 2018, 13 of 25 existing groups were followed and in 2019, 9 of 11 horse groups were followed. Two groups (Gozen&Nagaoka and Takaoka&Uozu) and 18 out of 84 individuals were followed in both years (Table 1). During a 1 h group focal sampling session, social behaviors (i.e., grooming and agonistic events) were documented using all occurrence sampling method and all individuals were recorded continuously with a video camera (Sony HDR-CX500V, Japan). The behaviors and names of all individuals were dictated to the camera to facilitate future video coding. Simultaneously, aerial photos were taken using an unmanned aerial vehicle (drone, Mavic PRO, DJI, China) to examine the horses' spatial positioning and inter-individual distance (see details in the next section). In this study, only data on groups that were followed for at least 8 h were analyzed, and observations in which the group composition changed during the target breeding season were excluded (i.e., the integration or disappearance of group members). However, because two-male groups were underrepresented in our dataset, an exception was made and a group with 6 h of observation was included in the analyses. Thus, a total of 16 groups and a total observation time of 193 h were included in this study (Table 1).

2.2.2. Spatial data

A drone was used to take aerial photos of each focal group (Fig. 1.). At the same time, a researcher conducted scan sampling to record the spatial positioning of individuals in order to facilitate individual identification in the aerial photos. Thus, each photo taken by the drone corresponded to a scan. To avoid data pseudoreplication, the scan interval for the capture of aerial photos was set at 10 min. According to previous studies, 10 min is an appropriate interval to assume data independency (Feh, 1988, Christensen et al., 2002) because the probability of retaining the same nearest neighbor drops drastically after 8 min (Wells and Feh, Unpublished, quoted by Feh, 1988). The drone was operated remotely with the camera angle set vertically downwards facing the ground, and the focal group was positioned in the center of the frame. The drone took off approximately 10–50 m from the horses and typically flew at an altitude of 65 m, however the height varied from 30 to 90 m depending on weather conditions (e.g., presence of fog) and spread of the group. Previous test flights confirmed that horses did not show a response towards the drone if it maintained a distance of at least 10 m (Inoue et al., 2018).

2.3. Defining and describing response variables

2.3.1. Inter-individual distance

The photos taken by a drone were treated following the protocol developed by Inoue et al., 2018, Inoue et al., 2020 to determine the location of the individuals and inter-individual distance. An individual's location was set as the midpoint between the tip of the head and the base of the tail. Body length (BL) was defined as the distance between the base of the tail to the base of the neck, which was relatively constant and straight. For each photo, the average BL of all individuals in the focal group, excluding foals, was calculated and used as a unit of length to measure the distance between individuals. This procedure was used because the height of the drone varied from scan to scan, and thus the scale of the images was different in each photo (Inoue et al., 2018). Since we only analyzed photos in which all group members were present (excluding photos where horses were located under trees and/or where at least one individual was

outside of the frame), the average body length of all individuals should remain consistent across scans.

In total, 1204 photos ranging from 3 to 7 photos taken during a one-hour period, were analyzed. Thus, the number of photos per group do not exactly reflect the total number of hours the group was observed (Table 1).

2.3.2. Proximity

Two individuals were defined as being in proximity with each other if they were within 3 BL from one another. The 3 BL threshold was set because it measures the distance between the center point of two individuals, not from the edge of one individual to the edge of another as in previous studies using 2 BL (van Dierendonck et al., 2004; Cameron et al., 2009). So, in the current study, the "radius" (half-width) of the two individuals, approximated as 0.5 BL was added to the previous threshold (2 BL). Hence, 3 BL (0.5 BL + 2 BL + 0.5 BL) is the newly defined threshold, nearly equivalent to the 2 BL threshold used in previous studies (Fig. 2.). However, it is possible that 3 BL in the current study is slightly overestimated when horses stand parallel to each other compared to when they face each other. Nonetheless, this method provides a more accurate and objective measurement of inter-individual distance because it does not rely on observers judgment.

2.3.3. Grooming behavior

Grooming was defined as reciprocal coat care where two partners stand beside one another, often head-to-shoulder or head-to-tail, and groom each other's neck, mane, rump or tail by gently nipping, nuzzling or rubbing (Christensen et al., 2002, McDonnell, 2003). A grooming event was initiated when one of the individuals touched the neck, mane or rump of another individual, who then immediately reciprocated this behavior; and ended when one of the individuals stopped the behavior. All grooming events recorded between individuals older than one year were mutual (hereafter, mutual grooming is referred to as grooming). Unidirectional grooming events were observed only by foals towards their mothers and other group members, and this data was not included in our analyses.

2.4. Social predictors

2.4.1. Sex

To test for the effect of sex homophily, all possible intra-group dyads were either classified as "same-sex" dyads if they were composed of same-sex individuals (female-female and male-male) or "different-sex" dyads if the dyads were composed of different-sex individuals (female-male). Due to the discrepancy between the number of female-female (n = 143) and male-male (n = 5) dyads, same-sex dyads were grouped into the same class. In total, 148 same-sex dyads and 91 different-sex dyads were observed.

2.4.2. Rank

Agonistic behaviors including displacements, mild threats, bite threats, kick threats, bites, kicks with the hind legs and strikes with the forelegs were recorded using all occurrence sampling (McDonnell, 2003, Christensen et al., 2002). In total, 1651 agonistic behaviors were recorded in the 16 groups during the 2018 and 2019 breeding seasons. To construct the winner/loser matrices to assess dominance hierarchy, all agonistic events were combined and only the behaviors that elicited a submissive response (avoidance or withdrawal) were considered (Wells and von Goldschmidt–Rothschild, 1979, Feh, 1999). Winner/loser matrices were built for each horse group and an individual's rank within a group was determined by assessing David's Score (DS) (David, 1987). DS produces a score for each individual based on the observed number of wins and losses within a dyad in agonistic encounters. To calculate DS, the 'steepness' R package was used (de Vries et al., 2006). First, the proportion of wins by individual i when interacting with an individual j (Pij) was calculated as the number of times that i defeats j (sij) divided by the total number of interactions between i and j (nij):

$$P_{ij} = \frac{s_{ij}}{n_{ij}}$$

Second, the DS for each group member, i, was calculated as:

$$DS = w + w_2 - l - l_2$$

Here, w represents the sum of i's Pij values and w2 represents the weighted sum of i's Pij (weighted by the w values of individuals interacting).

Likewise, l represents the sum of i's Pji values, and l2 represents the weighted sum of i's Pji values (weighted by the l values of individuals interacting; David, 1987, de Vries et al., 2006). In order to allow for comparison between different groups, DS' was standardized following the formula:

$$DS_i' = \frac{DS_i - DS_{max}}{DS_{max} - DS_{min}}$$

where DSi' values represent the individual's score within a group and DSmax and DSmin are the highest and lowest scores from individuals within a group, respectively. Values produced range from 0 to 1 for each individual (0 corresponding to the most subordinate and 1 to the most dominant individual). The absolute value of the difference between the DSi' of two individuals within a group was used for the analyses.

2.4.3. Familiarity

Familiarity was defined based on the amount of time individuals spent as group members, contrary to previous studies which characterized familiarity based on the location of individuals before joining the herd (i.e., unfamiliar individuals were from different herds that had not seen each other before the study; van Dierendonck et al., 2004; Pinter-Wollman et al., 2009, but see: Monard and Duncan, 1996). It is likely that individuals from the same population who share overlapping home ranges show a certain degree of familiarity towards each other, despite belonging to different groups (Maeda et al., 2021). So, varying degrees of familiarity between individuals were considered and classified into two different classes: "familiar" or "new". A "familiar" dyad consisted of individuals that were observed for at least one year in the same group. Conversely, a "new" dyad referred to individuals that were observed together for the first time in the breeding season that the observation took place. Because the study was conducted during the breeding seasons of 2018 and 2019 and population censusing was carried out occasionally during the non-breeding season, it was not possible to precisely determine when integration into new groups occurred

and therefore not possible to know the exact durations of social relationships. For that reason, the threshold for "new" dyads was set as individuals who have been observed together for less than one year (Supplementary material S1).

2.4.4. Genetic relatedness

2.4.4.1. DNA extraction, markers and genotyping

During the breeding seasons of 2017–2020, between May and August, 269 fecal samples were collected from visually identified horses. About 1–2 g of the outer layer of feces was collectedimmediately after the visual identification of the individual that defecated. The samples were stored in vials with 25 mL of 96% ethanol and placed in a cooling bag right after collection. Finally, the fecal samples were preserved in a freezer at a temperature of $-20\ °$ C. DNA extraction from fecal samples followed the GuSCN/silica protocol of Frantz et al. (2003). Potential PCR inhibitors were removed from DNA extracts using pre-rinsed Microcon® YM-30 centrifugal Filter Units (MILLIPORE). Individual identification was achieved using a set of 11 horse microsatellite markers (AHT4, ASB2, ASB17, ASB23, HMS1, HMS2, HMS3, HMS6, HMS7, HTG6 and VHL20) matching the recommended core or extra panels for individual genotyping of horses by the International Society for Animal Genetics (ISAG 2017). Microsatellites were amplified in two-steps using a pre-amplification protocol (Smith et al., 2011). In both steps, markers were pooled in two multiplex sets with six (MS1) and five (MS2) markers, respectively, and amplified in 10 μ L final volume reactions using the Multiplex PCR Kit (QIAGEN). Four replicas of each PCR step were performed to minimize amplification errors. Details for markers, multiplex sets and thermocycling conditions are given in the Supplementary material S2 and S3. Fluorescent labeling of PCR fragments was accomplished following Blacket et al. (2012). PCR products were separated by size on an ABI3130xl genetic analyzer. Alleles were scored against the GeneScan500 LIZ size standard, using GENEMAPPER 4.1 (Applied Biosystems) and checked manually by two observers. All lab procedures were conducted under sterile conditions and positive air pressure in dedicated rooms, and negative controls were included

throughout manipulations to monitor possible DNA cross-contaminations. Consensus genotypes over replicas were assembled manually following Godinho et al. (2015): i) heterozygous genotypes were accepted if the same genotype was observed in two independent PCRs; and ii) homozygous genotypes were accepted if the genotype was observed in three independent PCRs. Samples with more than 20% missing data were excluded. Identical genotypes were filtered using GIMLET 1.3.2. (Valière, 2002).

2.4.4.2. Analyzing genetic relatedness

Genetic relatedness is defined as the probability of two individuals sharing a gene. For example, the probability that two siblings share the same gene by descent is 0.5 and for cousins is 0.125 (Nowak, 2006). Pairwise genetic relatedness (r) was calculated between 178 individuals using the Triadic likelihood estimator (TrioML) implemented in software COANCESTRY 1.0 (Wang, 2011), with 10,000 bootstraps and allele frequencies of 83 individuals from the Serra d'Arga Garrano horse population. This likelihood method uses the genotypes of a third individual as a reference to estimate the r between two focal individuals, therefore reducing the chance of genes identical in state being mistakenly inferred as identical by descent (Wang, 2007). To select the best relatedness estimator, 83 genotypes were simulated based on the empirical allele frequencies of non-closely related individuals, missing data and error rates for the 11 microsatellite markers genotyped from Serra d'Arga horses. Subsequently, the relatedness of dyads with known relationship was compared to their true relatedness coefficient for seven estimators (see Supplementary material S4). The empirical TrioML estimate values, ranging from 0 to 1 for each dyad, were considered for the analyses to investigate the effect of genetic relatedness on affiliation and group cohesion.

2.4.5. Group stability and size

Group stability was assessed considering the proportion of individuals leaving the group (including individuals that disappeared) and the proportion of individuals integrating into the group (Table 2). The proportion of individuals that left the group (PT) was calculated by dividing the number of individuals

(males or females) that transferred/disappeared during the interval between the two breeding seasons (2017–2018 and 2018–2019) by the number of individuals existing in the group in the previous breeding season. The proportion of individuals that integrated into the group (PI) was calculated by dividing the number of individuals (males or females) that immigrated into the group during the interval between the two breeding seasons by the number of individuals existing in the group during the observed breeding season. For a group to be considered "stable", the proportion of individuals who transferred/disappeared or integrated into the group had to be ≤ 0.5 (Table 2; i.e., the number of individuals who left or integrated into the group was lower than the number of individuals who composed the core of the group). Conversely, if the proportion of individuals who transferred/disappeared or integrated into the group was > 0.5, then the groups would be classified as "new". Following our definition of stability, seven groups were categorized as "new" and nine groups as "stable". Group size counted all individuals older than one year of age. The average group size of our focal groups varied from 6.56 ± 0.92 (mean ± SD, n = 9) in 2018–4.78 ± 0.67 (mean ± SD, n = 9) in 2019.

2.5. Statistical analysis

Generalized linear mixed models (GLMMs) were built to investigate the influence of social factors on inter-individual distance, dyadic proximity and grooming behavior using the R package 'glmmTMB' (R × 64 3.5.0, https://www.Rproject.org; Brooks et al., 2017). This package allowed for the incorporation of the covariance structure AR (1) to control for serial autocorrelation in the residuals, and made it possible to run more complex models, for example, to test zero inflated data. The autocorrelation factor (+ ar1 (times + 0 | group), where times = scan, group date) was considered in the proximity and inter-individual distance models in which the AIC (Akaike Information Criterion) was significantly lower. We reported the models with the lowest AIC that incorporated the variables to be tested: sex, rank difference, familiarity, group stability, relatedness and group size. After a stepwise selection procedure, only the models in which the interaction between the social factors

and stability improved the fit of the model (given by the lower AIC) were considered. Additionally, the models were checked for inter-correlation of the predictors by examining multicollinearity using variance inflation factors (VIF; values less than 5, which indicate low correlation, were acceptable). One individual was not sampled for genetic analyses, therefore the genetic relatedness of the dyads including this individual (N = 5) were excluded from the analyses. In total, 234 of the 239 dyads were analyzed.

2.5.1. Inter-individual distance model

A GLMM with a Gaussian error structure was built to investigate the influence of social factors on inter-individual distance. The data on inter-individual distance in BL was subjected to a logarithmic transformation in order to normalize the value of the residuals and fit a GLMM with a Gaussian error structure. Sex, rank difference, familiarity, group stability, genetic relatedness and group size were included as fixed effects. In this model, the interaction between group stability and sex, as well as between group stability and rank were added because they improved the model's fit (lowering the AIC). The identity of the two horses involved in the interaction, group identity and the date of observation were included as random effects to control for repeated measures. In this model, a total of 15,822 data points and 234 dyads were examined.

2.5.2. Proximity model

A GLMM with a binomial error structure (for each scan, dyads within a distance of 3 BL were scored as 1 and dyads with a distance greater than 3 BL were scored as 0) and logit link function was built to investigate the social factors influencing proximity. Sex, rank difference, familiarity, group stability, genetic relatedness and group size were included as fixed effects. The identity of the two horses, group identity and date were included as random effects. The interaction between group stability and rank difference was also included. In this model, a total of 15,822 data points and 234 dyads were examined.

2.5.3. Grooming model

To assess which factors influence grooming frequency between dyads, a GLMM with a zero inflated Poisson structure for the counts of grooming events, including a logit link function was built. A zero inflated model was selected because of the zero skewed nature of the data, which could be due to the presence of structural zeros, related to ecological and behavioral restrictions of the species and/or random zeros resulting from sampling variability (Blasco‐Moreno et al., 2019). Using 'glmmTMB' allowed us to include a ziformula in the model, which describes how the probability of a structural zero will vary according to the predictors (Brooks et al., 2017). A single zero-inflation parameter was applied to all observations (ziformula ~ 1), as no initial assumptions about the variation of absences, i.e. structural zeros, were established (e.g., absences could vary according to sex or familiarity of the dyads). Sex, rank difference, familiarity, group stability, genetic relatedness and group size were included as fixed effects in the model. The identity of both horses, group identity and date were included as random effects, as in the previous models. In this model, a total of 2567 data points and 234 dyads were examined.

3. Results

3.1. General social predictors patterns

The dyads analyzed consisted of 89 (38%) possible intra-group "different-sex" dyads and 145 (62%) intra-group "same-sex" dyads, of which 140 (97%) were female-female dyads and 5 (3%) were male-male dyads (Supplementary material S5). Since some groups and dyads were observed during two consecutive breeding seasons (2018–2019) and, at dyadic level, social factors may have changed from one year to the following, averages are reported separately for both breeding seasons (BS). In the BS of 2018, 116 (62%) dyads were classified as familiar and 52 (31%) as new dyads, whereas in the BS of 2019 the percentage of new dyads increased to 70% (46 of 67 dyads). In total, 134 (57%) were classified as familiar and 101 (33%) as new dyads (Table S5). The category of only two dyads changed from the BS of 2018 to the BS of 2019, from new to familiar.

The social rank based on standardized David's Score (DSi') obtained for each individual and the difference between the DSi' of two individuals for each dyad is shown in the Supplementary material S6 and S7. The likelihood of observing a female in the highest ranking position according to the sex ration is 0.75, while for males is 0.25 in 2018 ($\chi 2$ = 13, df = 1, P = 0.003). In 2019, the likelihood is 0.73 for females and 0.27 for males ($\chi 2$ =8.80, df=1, P = 0.003). Overall, females tend to occupy the highest ranking position (DSi' = 1) in 12 (80%) out of 16 groups. However, the lowest ranking position was never occupied by males. Natal young females and young females which have dispersed to new groups were always the lowest ranking individual of their groups (DSi' = 0). On average males showed higher DSi' (BS 2018: 0.64 $\pm$ 0.25, n = 14; BS 2019: 0.77 $\pm$ 0.29, n = 11) compared to females (BS 2018: 0.51 $\pm$ 0.33, n = 45; BS 2019: 0.50 $\pm$ 0.39, n = 32).

The intra-group dyads in this population were fairly closely related (n = 234), since 46 (20%) of the dyads had a value of r $\geq$ 0.125, and only 23 (10%) had a value of r $\geq$ 0.50 (Supplementary material S7). The mean relatedness per group was 0.08 $\pm$ 0.04 (mean $\pm$ SD, n = 8) in the BS of 2018, 0.07 $\pm$ 0.05 (n = 8) in the BS of 2019.

3.2. Influence of social factors on inter-individual distance and group cohesion

The average inter-individual distance for all intra-group dyads was 9.34 $\pm$ 2.56 BL (n = 168) in the BS of 2018 and 9.91 $\pm$ 3.89 BL (n = 85) in the BS of 2019. The distribution of the observed intra-group dyadic distance and the random intra-group dyadic distance differed significantly according to the Kolmogorov–Smirnov test (P < 0.001, Fig. 3A).

Sex homophily had a negative effect on inter-individual distance; same-sex dyads maintained shorter distances compared to different-sex dyads, both in stable and new groups (GLMM: Z = $-$8.26, P < 0.001, Table 3, Fig. 4.). Rank difference had no effect on inter-individual distance (GLMM: Z = 0.85, P = 0.40, Table 3), however the interaction between group stability and rank difference showed an opposite trend between stable and new groups; inter-individual distance

increased with greater rank difference in new groups and decreased in stable groups (GLMM: Z = −2.90, P = 0.004, Table 3, Fig. 4.). Familiar individuals maintained shorter distances compared to new dyads (GLMM: Z = −3.15, P = 0.002, Table 3, Fig. 4.) and closely related horses maintained greater distances between them (GLMM: Z = 2.41, P = 0.02, Table 3, Fig. 4.). Inter-individual distance was not predicted by the stability of the group (GLMM: Z = 0.26, P = 0.79, Table 3) and increased with a larger group size (GLMM: Z = 2.83, P = 0.005, Table 3).

3.3. Influence of social factors on proximity

In the BS of 2018, 162 (96%) dyads were observed in proximity (< 3 BL) at least once and each dyad spent on average 20 ± 12% (n = 168) of the scans in proximity. In the BS of 2019, 83 (98%) dyads were observed in proximity, spending on average 22 ± 15% (n = 85) of the scans in proximity. When considering only the intra-group dyadic distances < 3BL (the threshold for proximity), the Kolmogorov–Smirnov test revealed a significant difference between the observed proximity distribution and the random proximity distribution (P < 0.001, Fig. 3B).

Sex homophily had a positive effect on proximity; same-sex individuals were in proximity more often than different-sex pairs (GLMM: Z = 8.67, P < 0.001, Table 3, Fig. 5.). Rank difference had a marginal negative effect on proximity (GLMM: Z = −1.65, P = 0.10, Table 3, Fig. 5.) and the interaction between group stability and rank difference showed that proximity decreased with increasing rank difference in new groups, while the opposite pattern was observed in stable groups (GLMM: Z = 2.44, P = 0.01, Table 3, Fig. 5.). Familiarity had a positive effect on proximity; familiar individuals were in proximity more often compared to individuals with a new bond (GLMM: Z = 4.38, P < 0.001, Table 3, Fig. 5.); while proximity decreased with increasing genetic relatedness (GLMM: Z = −2.74, P = 0.006, Table 3, Fig. 5.). The stability of the group did not have an effect on proximity (GLMM: Z = −0.95, P = 0.34, Table 3) and the frequency of individuals in proximity decreased with increasing group size (GLMM: Z = −2.09, P = 0.04, Table 3).

3.4. Influence of social factors on grooming

In a total of 193 h of focal group observations, 155 grooming events (0.80 events/h) were recorded. In the BS of 2018, grooming was observed in 41 (24%) of the dyads analyzed and the average grooming frequency per dyad was 0.06 ± 0.12 events/h (n = 168). In the BS of 2019, grooming was observed in 32 (38%) of the dyads analyzed with an average grooming frequency per dyad of 0.08 ± 0.14 events/h (n = 85). Sex and rank difference, as well as genetic relatedness, had no effect on grooming frequency (sex: GLMM: $Z = 0.59$, $P = 0.55$; rank: $Z = -0.82$, $P = 0.41$, genetic relatedness: $Z = 1.59$, $P = 0.11$, Table 3, Fig. 6.). Familiarity had a positive effect on grooming frequency; grooming was more likely to occur in familiar rather than new dyads (GLMM: $Z = 2.88$, $P = 0.004$, Table 3, Fig. 6.). Group stability did not predict grooming frequency (GLMM: $Z = -1.37$, $P = 0.17$, Table 3, Fig. 6.) and grooming frequency decreased with increasing group size (GLMM: $Z = -3.02$, $P = 0.00$

4. Discussion

Our predictions about the effect of sex and rank on affiliation were only partially supported, while the predictions on the effect of familiarity and genetic relatedness on affiliation were supported by our results. Overall, our findings suggest that sex homophily, and more notably familiarity, played a fundamental role in fostering intra-group bonds. This is in line with previous studies in both free-ranging and domestic settings (Sigurjónsdóttir et al., 2003, van Dierendonck et al., 2004, Heitor et al., 2006b, Bouskila et al., 2016, Stanley et al., 2018), despite these feral horses being subjected to wolf predation and anthropogenic interference. Regarding group level factors, our results showed that inter-individual distance and affiliation were not affected by group stability, other than when interacting with rank. Individuals in larger groups tended to be less cohesive, given the greater inter-individual distance, spent less time in proximity and groomed less often. Social predictors showed a similar, however opposite, trend in both inter-individual distance and proximity models, suggesting that inter-individual distance can also be used as a proxy for proximity.

4.1. Sex homophily

The tendency for same-sex dyads to maintain shorter distances likely originated from differences in the quality of social relationships and ecological needs of each sex (Sueur et al., 2011). Males tend to occupy more peripheral positions in harem groups, as they are responsible for group defense against outgroup male harassment (Berger, 1986; Linklater, 1999; Feh, 1999; Inoue et al., 2018), while females tend to be located more centrally in the group while grazing (Inoue et al., 2018). A central position in the group would offer females and their respective offspring better protection against harassment and predation, as individuals located in the center of the group are less likely to suffer predator attacks, especially in a high predation risk environment (Moscovice et al., 2020). Cohesion among females may also be driven by male herding behavior (Ringhofer et al., 2020). However, this behavior alone may not be sufficient to explain the greater proximity existing between females, as no association between herding behavior and reformations or new formations of harem groups was found (Kaseda and Khalil, 1996). Greater levels of affiliation between same-sex conspecifics have been consistently reported in studies of horses (Sigurjónsdóttir et al., 2003, van Dierendonck et al., 2004, Bouskila et al., 2016) and of other mammalian taxa (e.g. spotted hyenas, Crocuta crocuta: Smith et al., 2007; primates species: Sterck et al., 1997; African elephants, Loxodonta africana: Lee, 1987; plains zebras, Equus quagga: Tong et al., 2015). Our findings revealed similar patterns, namely that same-sex dyads spent more time in proximity compared to different-sex dyads, suggesting that individuals may be benefiting from these affiliations. A preference for female horses to affiliate with each other may exist to offer better protection against male aggression (Linklater et al., 1999, Cameron et al., 2009). Females who maintain strong social bonds also experience increased reproductive success both in feral horses (Cameron et al., 2009) and primate species (Silk, 2007). Male-male bonds in multiple-male harem groups may be essential to foster alliances useful for maintaining group cohesion, ultimately enhancing their inclusive fitness (Feh, 1999). In other mammalian species (e.g. bottlenose dolphins, Tursiops truncatus, and Barbary macaques, Macaca sylvanus) males have been reported to form intra-sexual

alliance to gain access to fertile females (Connor et al., 2001; Bissonnette et al., 2011).

Despite the clear preference for same-sex conspecifics to remain closer in proximity, they do not groom more often. The relationship between grooming and proximity is not consistent across horse studies, as proximity is not always associated with grooming behavior (Roberts and Browning, 1998, Kimura, 1998, Inoue et al., 2018 but see: van Dierendock et al., 1994; Sigurjónsdóttir et al., 2003; Shimada and Suzuki, 2020). Close proximity between conspecifics could be the result of either individual initiatives (e.g. grooming) or of passive acceptance (van Dierendock et al., 2004). Although we did not investigate the direct relationship between grooming and proximity, our results support the hypothesis that proximity may be a result of passive acceptance, rather than an active process in this feral population. Grooming may reduce social tension (Spruijt et al., 1992), stress levels (Feh and de Mazieres, 1993) and strengthen social bonds amongst group members (horses: Kimura, 1998; Feh, 1999; Shimada and Suzuki, 2020; primates: Seyfarth, 1977; Silk, 2007; Seyfarth and Silk, 2014). Grooming occurred at similar rates between same- and different-sex dyads in this population of feral horses, which might reflect the importance of maintaining both strong intrasexual and intersexual bonds to preserve group cohesion. Differences found related to the effect of sex homophily on grooming and proximity may suggest that these two behaviors act as different mechanisms to promote cohesion among conspecifics. If grooming represents a stronger affiliative behavior than proximity as previously suggested (Roberts and Browning, 1998), individuals that groomed each other more often would not necessarily need to be in constant proximity. Therefore, as males in this study were not able to remain in constant proximity with females, engaging in grooming may have been a way to maintain intersexual bonds.

4.2. Rank homophily

Affiliation among conspecifics of a similar hierarchical rank status to their own has been described in horses (Clutton–Brock et al., 1976, Wells and von Goldschmidt–Rothschild, 1979, Kimura, 1998; Sigurjónsdóttir et al.,

2003; Briard et al., 2015; but see: van Dierendonck et al., 1995; Heitor et al., 2006b) and in various mammalian species (red deer, Cervus elaphus: Appleby, 1983; rhesus monkeys, Macaca mulatta: de Waal, 1991; chacma baboons, Papio hamadryas ursinus: Seyfarth et al., 2014). This pattern may be explained by the greater intensity of aggression directed towards lower ranking individuals by higher ranking individuals, which causes them to move further away from dominant individuals (Heitor et al., 2006a). In our study, being of similar rank did not predict affiliation among group members. In new groups, individuals of similar rank status maintained shorter distances and spent more time in proximity compared to individuals with greater rank differences, in line with other reports (Kimura, 1998, van Dierendonck et al., 2004, Heitor et al., 2006b). However, in stable groups the opposite pattern was observed. Higher ranking horses may be showing more tolerance, suggested by the closer proximity, towards lower ranking conspecifics due to the existence of a more stable hierarchy. Hierarchies in stable horse groups tend to be relatively constant over time (Tyler, 1972; van Dierendonck et al., 1995; Sigurjónsdóttir et al., 2003) and are correlated with group stability (Granquist et al., 2012). Whereas when group composition changes, it may take time for a new hierarchy to develop because each dyad must establish a dominance relationship (Granquist et al., 2012). Thus, we propose that maintaining closer proximity with individuals of similar rank might be a strategy to avoid aggression from higher ranking individuals in newly formed groups where a new hierarchical structure might be under development.

4.3. Familiarity and genetic relatedness

The potential influence of familiarity on affiliation patterns has not been thoroughly investigated, as most previous studies targeted groups or herds of horses that were already familiar with each other (e.g., Heitor et al., 2006b; Bouskila et al., 2016). Studies which addressed this reported that horses tend to affiliate with familiar individuals after the transfer of unfamiliar horses into the group (van Dierendonck et al., 2004) and that familiarity may affect young females' choice during dispersal, as they tend to disperse to groups with

familiar females while avoiding related males (Monard and Duncan, 1996). Additionally, male partners who formed alliances to hold a harem together were reported to be of similar age and were close associates in their natal group, therefore they were familiar to one another, but they were not closely related to each other (Feh, 1999). In our study, familiarity predicted not only shorter distances and more time spent in proximity between individuals, but also higher grooming frequencies in both stable and new groups. Shorter inter-individual distance and proximity among group members were not predicted by genetic relatedness, and no effect was found for grooming. This suggests that these feral horses did not tend to affiliate with closely related individuals, corroborating with previous observations where relatedness was not a determinant factor of affiliation (Clutton–Brock et al., 1976, van Dierendonck et al., 1995, Heitor and Vicente, 2010, Bouskila et al., 2016); and where a preference for affiliating with familiar conspecifics exceeded the preference for related individuals (Linklater, 2000, Monard et al., 1996; van Dierendonck et al., 2004). Our findings may be explained partially by the horses' bisexual natal dispersal pattern (Tyler, 1972; Monard et al., 1996; Kaseda et al., 1997). Dyads observed in this study were composed mostly of individuals older than 2-years-old (with the exception of a 1-year-old female) who tended to associate with group members other than their mothers and had fewer opportunities to bond with older siblings, as they had dispersed (Mendonça, unpublished results). The absence of interaction between sex homophily and genetic relatedness in the models exposed a similar pattern for same- and different-sex dyads, suggesting that different-sex dyads tended to maintain greater distances if they were related. Spatial avoidance between related males and females (which consisted mainly of father-daughter and full-sibling pairs) would be expected, considering that young females avoid mating with familiar or related males from their natal group as a strategy to avoid inbreeding (Berger and Cunningham, 1987, Monard et al., 1996).

Differing behavior towards genetically related individuals raises the question of whether horses possess kin recognition mechanisms. Unlike some other mammals, horses are unable to recognize kin by their odors via the major histocompatibility complex, a putative kinship marker (Brown and Eklund,

1994, Green et al., 2015, Jaworska et al., 2020). Instead, the tendency to interact with closely related individuals may be due to an "ancillary kin bias" (Heitor et al., 2006a), i.e., individuals may demonstrate differential behaviors towards kin versus non-kin due to factors that are arbitrarily correlated with kinship (Grafen, 1990, Tang–Martinez, 2001). Familiarity is one such factor as it is often a confounding variable for relatedness (e.g., Monard and Duncan, 1996; Sigurjónsdóttir et al., 2003). Early association between individuals in their natal group prior to their dispersal may be a mechanism for kin recognition (Berger and Cunningham, 1987). This could potentially explain the preference for females to interact with kin (Sigurjónsdóttir et al., 2003, Gilbert-Norton et al., 2004, Heitor et al., 2006b) and their tendency to avoid related males prior to and after dispersal (Feist and McCullough, 1976, Monard and Duncan, 1996). Affiliative behaviors directed towards kin have been reported across several mammalian taxa (e.g. wedge-capped capuchin, Cebus olivaceus: O'Brien and Robinson, 1991; yellow baboons, Papio cynocephalus: Silk et al., 2006; sperm whales, Physeter microcephalus: Gero et al., 2008; white-faced capuchins, Cebus capucinus: Perry et al., 2008; captive degus, Octodon degus: Jesseau et al., 2009; spotted hyenas: Crocuta crocuta: Smith et al., 2010; bottle nose dolphins, Tursiops truncates: Diaz-Aguirre et al., 2020) and benefits may be provided to kin who affiliate in primate species (Silk et al., 2003, Charpentier et al., 2007). Conversely, in feral horses, affiliation between unrelated females appears to provide positive direct fitness consequences, as it increases foal birth rates and foal survival (Cameron et al., 2009). Therefore, it is likely that affiliation with kin might not be as advantageous for feral horses as it is for other mammalian species living in matrilineal societies (primates: Silk, 2002; dolphins, Tursiops aduncus: Wiszniewski et al., 2010).

5. Conclusion

Our study is the first to investigate affiliation preferences of multiple feral horse groups. Our findings revealed that familiarity between individuals was the most consistent factor determining affiliation and a key element for preserving group cohesion. The relationship between rank and affiliation, as well as between genetic relatedness and affiliation, was not entirely consistent with existing

studies. Differences regarding rank in stable and new groups may be explained in part by recent changes in the groups' social composition that could have affected their hierarchical structure; and the absence of affiliation preferences between closely related conspecifics may be due to the dispersal pattern of this feral population. Social predictors affected the two affiliation types (proximity and grooming) differently, suggesting they may have distinct functions for promoting cohesion among group members. While proximity may be crucial for maintaining bonds between females, grooming might be beneficial for reinforcing both intrasexual and intersexual bonds. We suggest that future studies integrate group level factors such as composition and stability into their analyses, and consider them for cross-study comparisons, as we found they have an effect on affiliation in addition to group cohesion. A caveat of this study is that it includes only two breeding seasons. Future studies should take place beyond the breeding season months to account for potential seasonal variations on affiliation patterns and group cohesion. Moreover, other conspicuous social factors such as developmental stage, personality and reproductive status which were not addressed in this study should be examined in the future at a population level in feral horses. Investigating the affiliation patterns of feral horses, namely in populations that are affected by anthropogenic factors and subjected to predation, might shed light on their behavioral plasticity such as how behaviors are adjusted in the face of adversity to preserve group cohesion. Ultimately, understanding the social dynamics of feral horses may be useful for improving management guidelines working towards the conservation of free-ranging and feral horse breeds, as well for improving the welfare of domestic horses in captivity.

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BS	Focal group	N_{BS}	N_{BS-1}	Group core[a]	N_T[b]	N_I[c]	P_T[d]	P_I[e]	Stability
2018	Gozen&Nagaoka	5	5	5 (3 f,2 m)	0	0	0.00	0.00	stable
	Hakata	5	5	4 (2 f,1 m,1yf)	1 m	1 f	0.20	0.20	stable
	Harajyuku	8	8	7 (6 f,1 m)	1 f	1 f	0.13	0.13	stable
	Hitachi	6	8	6 (3 f,1 m,1yf,1ym)	2 f	0	0.25	0.00	stable
	Kamakura&Zama	6	6	6 (4 f,2 m)	1 m	0	0.17	0.00	stable
	Kitakami	8	6	4 f	2 (1 f,1 m)	4 (3 f,1 m)	0.33	0.50	new
	Kobe	9	8	7 (6 f,1 m,1yf)	1 f	2 f	0.13	0.22	stable
	Nanba&Tennoji	7	4	3 (1 f,2 m)	1 f	4 f	0.25	0.57	new
	Takaoka&Uozu	4	4	4 (2 f,2 m)	0	0	0.00	0.00	stable
2019	Aso	3	7	2 f	5 (4 f,1 m)	1 m	0.71	0.33	new
	Daisen	5	3	3 (2 f,1 m)	1 f	2 f	0.33	0.40	stable
	Gozen&Nagaoka	4	5	2 (2 f,2 m)	1 f	0	0.20	0.00	stable
	Hirosaki	5	–	0	–	5 (4 f,1 m)	–	1.00	new
	Nanba	6	6	3 f	3 (2 f,1 m)	3 f	0.50	0.50	new
	Seki	4	2	2 (1 f,1 m)	0	2 f	0.00	0.50	new
	Takaoka&Uozu	5	5	5 (2 f,3 m)	0	0	0.00	0.00	stable
	Toki	5[f]	4	4 (2 f,1 m,1yf)	0	0	0.00	0.00	stable
	Unnan	6	5	3 f	3 (2 f,1 m)	3 (2 f,1 m)	0.60	0.50	new

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Fig. 1. Aerial photo taken with the drone to Unnan group (n = 6); each photo corresponds to a scan.
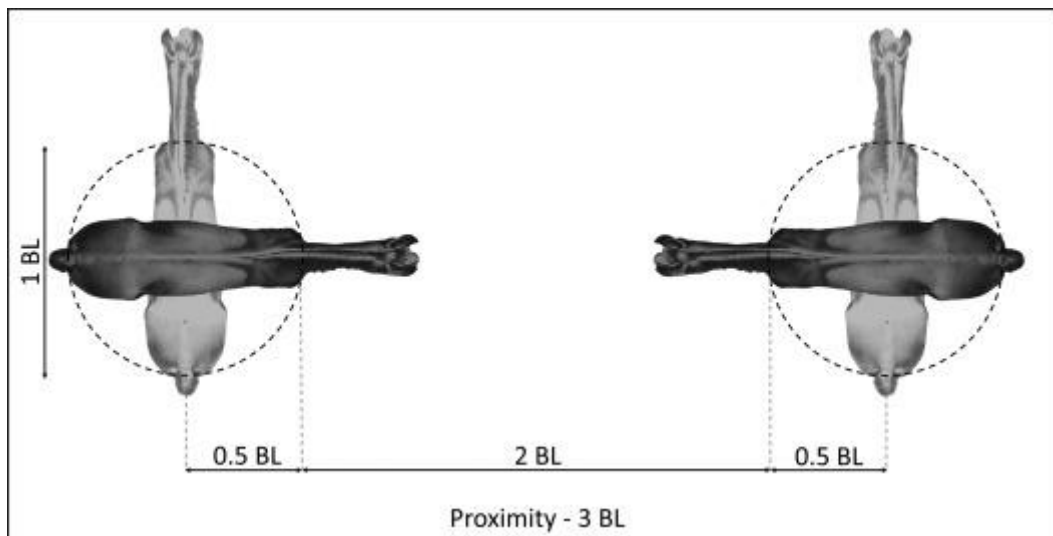

Fig. 2. Representation of the 3 BL proximity threshold, using 1 BL as the unit of length. The dark and light horses represent two possible orientations.
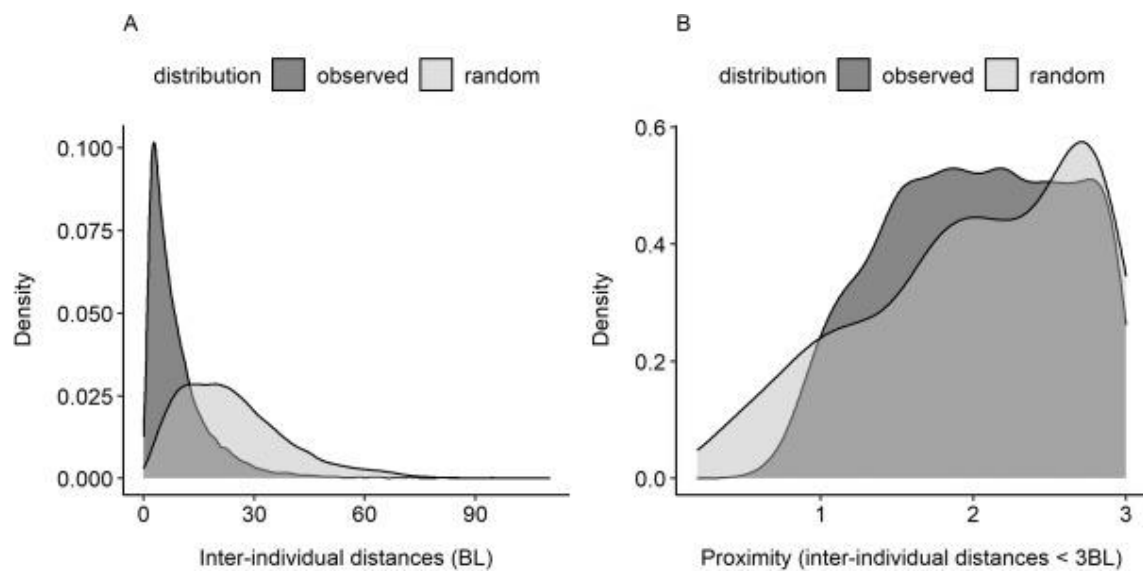
Fig. 3. Kernel density plot showing observed and random distributions for A) dyadic inter-individual distance and B) Proximity (inter-individual distance within 3 BL).
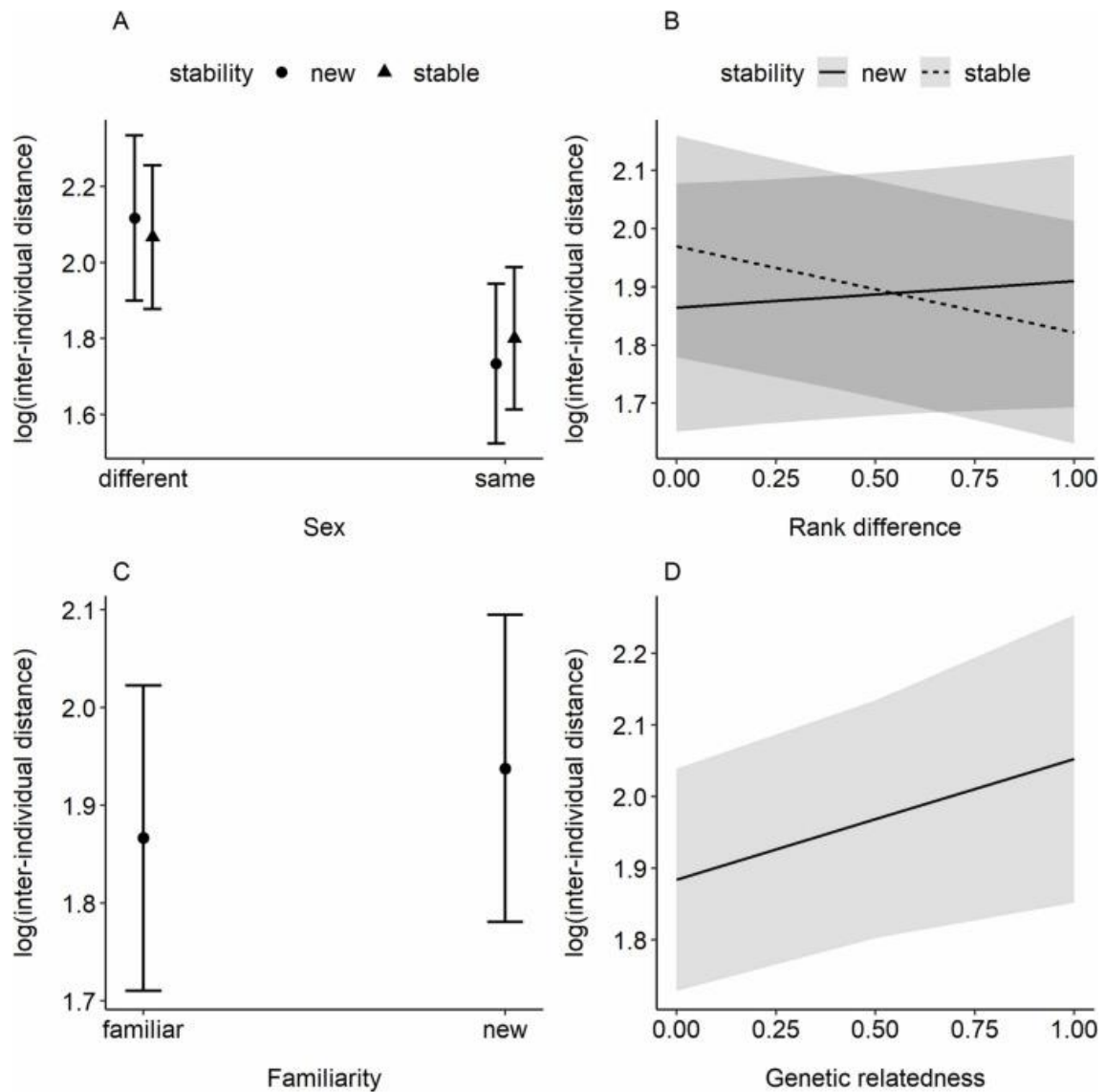
Fig. 4. Relationship between dyadic social predictors (A, B, C, D) and inter-individual distance in BL after a logarithmic transformation; A) interaction between sex and group stability, B) interaction between rank difference and group stability, C) familiarity, D) genetic relatedness. The values plotted correspond to predicted values (marginal effects) held constant at their proportions, bars represent the SE (standard error) and the shaded band represents the pointwise 95% confidence interval on the fitted value.
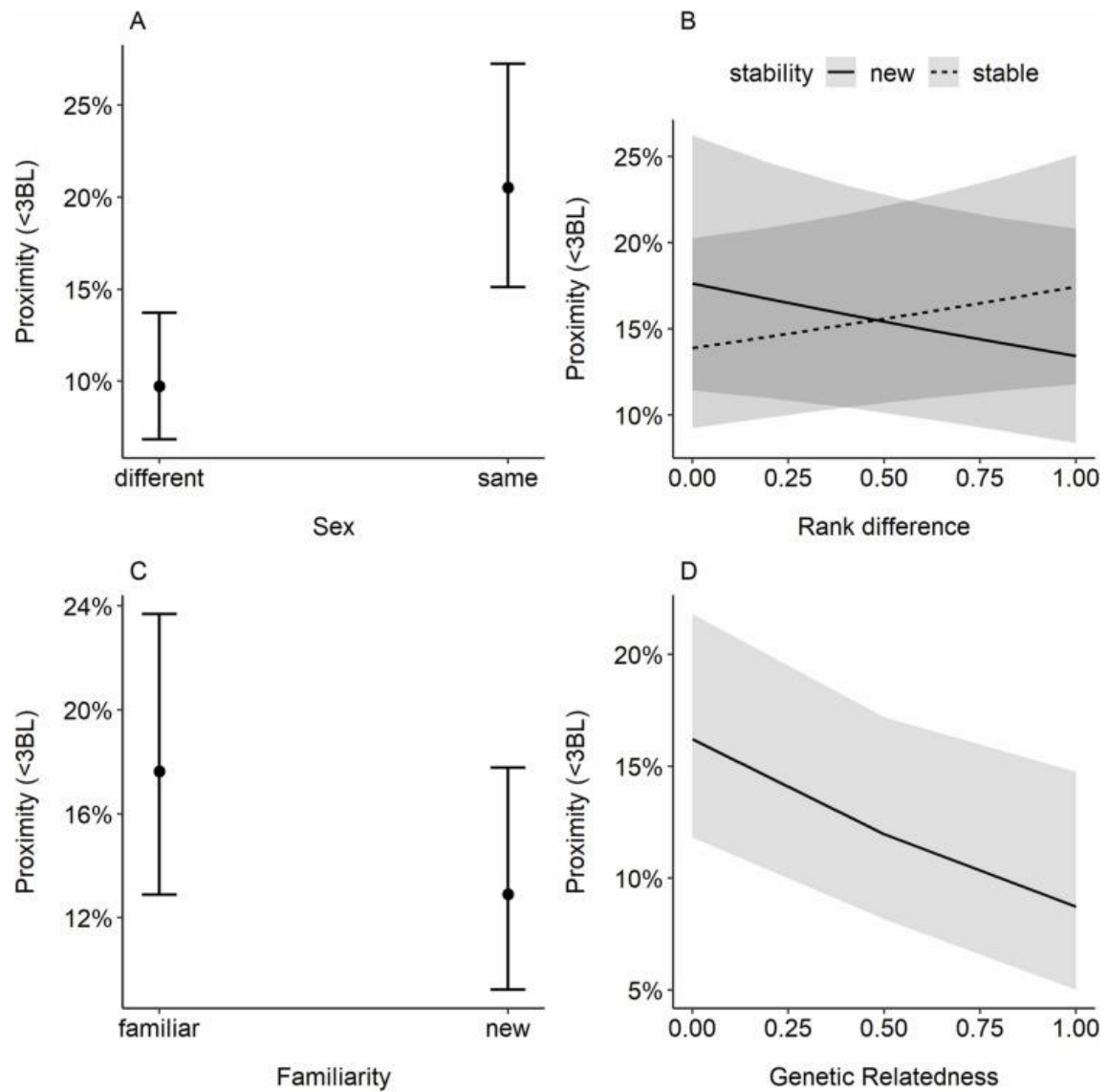
Fig. 5. Relationship between dyadic social predictors (A, B, C, D) and the % of scans spent in proximity (< 3 BL). A) sex, B) interaction between rank difference and group stability, C) familiarity, D) genetic relatedness. The values plotted correspond to predicted values (marginal effects) held constant at their proportions, bars represent the SE (standard error) and the shaded band represents the pointwise 95% confidence interval on the fitted value.

3, Table 3).

Fig. 6. Relationship between dyadic social predictors (A, B, C, D) and grooming frequency (events/h); A) sex, B) rank difference, C) familiarity, D) genetic relatedness. Only familiarity had a significant effect on grooming frequency (GLMM: Z = 2.88, P = 0.004). The values plotted correspond to predicted values (marginal effects) held constant at their proportions, bars represent the SE (standard error) and the shaded band represents the pointwise 95% confidence interval on the fitted value.
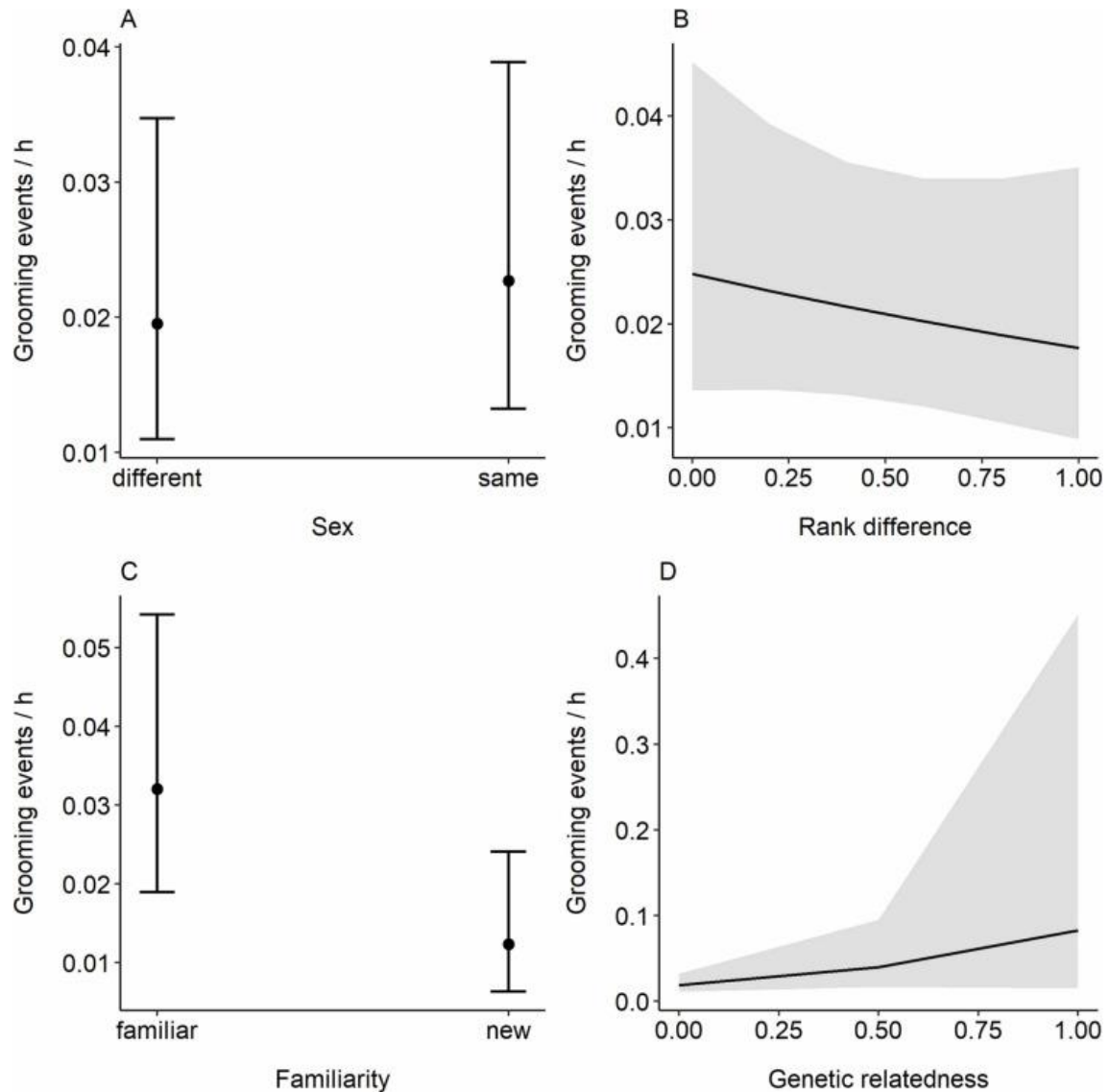
Year	Group name	Group size	N dyads	N Males	N females	N natal young individuals[a]	Hours observed[b]	Total of drone scans
2018	Gozen&Nagaoka	5	10	2	2	0	8	46
	Hakata	5	10	1	3	1	12	72
	Harajyuku	8	28	1	7	0	10 (12)	50
	Hitachi	6	15	1	3	2	8 (12)	51
	Kamakura&Zama	6	15	2	4	0	6 (10)	34
	Kitakami	8	28	1	7	0	10	67
	Kobe	9	36	1	7	1	8	60
	Nanba&Tennoji	7	21	2	4	0	9 (11)	46
	Takaoka&Uozu	5	10	2	2	1	10	59
	Total	59	173	13	39	4	81	485
2019	Aso	3	3	1	2	0	13	98
	Daisen	5	10	1	4	0	9	63
	Gozen&Nagaoka	4	6	2	2	0	15	94
	Hirosaki	5	10	1	4	0	13	70
	Nanba	6	15	1	5	0	13	80
	Seki	4	6	1	2	0	13	92
	Takaoka&Uozu	5	10	2	3	0	14	89
	Toki	5	10	1	3	1	9	60
	Unnan	6	15	1	5	0	13	77
	Total	43	85	11	30	1	112	723
2018–2019	Total	84	239	19	52	5	193	1208

Table 1

Summary of the data on the composition, observation time and total of drone scans for each focal group.

[a] Individuals younger than three years' old, males or females, that haven't dispersed from their natal groups.

[b] The number within parenthesis corresponds to the number of hours the group was followed; the number outside the parenthesis corresponds to the number of hours
used in the analyses. Data in which the group composition changed during the target breeding season were excluded.

BS	Focal group	N_{BS}	N_{BS-1}	Group core[a]	N_T[b]	N_I[c]	P_T[d]	P_I[e]	Stability
2018	Gozen&Nagaoka	5	5	5 (3 f,2 m)	0	0	0.00	0.00	stable
	Hakata	5	5	4 (2 f,1 m,1yf)	1 m	1 f	0.20	0.20	stable
	Harajyuku	8	8	7 (6 f,1 m)	1 f	1 f	0.13	0.13	stable
	Hitachi	6	6	6 (3 f,1 m,1yf,1ym)	2 f	0	0.25	0.00	stable
	Kamakura&Zama	6	6	6 (4 f,2 m)	1 m	0	0.17	0.00	stable
	Kitakami	8	6	4 f	2 (1 f,1 m)	4 (3 f,1 m)	0.33	0.50	new
	Kobe	9	8	7 (6 f,1 m,1yf)	1 f	2 f	0.13	0.22	stable
	Nanba&Tennoji	7	4	3 (1 f,2 m)	1 f	4 f	0.25	0.57	new
	Takaoka&Uozu	4	4	4 (2 f,2 m)	0	0	0.00	0.00	stable
2019	Aso	3	7	2 f	5 (4 f,1 m)	1 m	0.71	0.33	new
	Daisen	5	3	3 (2 f,1 m)	1 f	2 f	0.33	0.40	stable
	Gozen&Nagaoka	4	5	2 (2 f,2 m)	1 f	0	0.20	0.00	stable
	Hirosaki	5	–	0	–	5 (4 f,1 m)	–	1.00	new
	Nanba	6	6	3 f	3 (2 f,1 m)	3 f	0.50	0.50	new
	Seki	4	2	2 (1 f,1 m)	0	2 f	0.00	0.50	new
	Takaoka&Uozu	5	5	5 (2 f,3 m)	0	0	0.00	0.00	stable
	Toki	5[f]	4	4 (2 f,1 m,1yf)	0	0	0.00	0.00	stable
	Unnan	6	5	3 f	3 (2 f,1 m)	3 (2 f,1 m)	0.60	0.50	new

Table 2. Information on the focal groups' demography and membership changes occurred between the breeding seasons (BS) of 2018 and 2019) and the previous breeding seasons (BS-1; 2017 and 2018). f, females; m, males; yf, young females; ym, young males.

a

Group core – Number of individuals older than one year present during the two consecutive breeding seasons (2017–2018 and 2018–2019).

b

NT – Number of individuals transferred (or disappeared) during the interval between between the two.

c

NI – Number of individuals integrating the group during the interval between between the two.

d

PT – Proportion of individuals that left the group.

e

PI – Proportion of individuals that integrated into the group.

f

A foal became on year old and was included in the group composition.

Response	Fixed effects	Estimate	SE	X^2	Z	P
Log (inter-individual distance) in BL	(Intercept)	1.35	0.25	29.33	5.42	< 0.001
	Sex (same-sex)	-0.38	0.05	68.26	-8.26	< 0.001
	Rank difference	0.05	0.05	0.71	0.85	0.40
	Familiarity (familiar)	-0.07	0.02	9.92	-3.15	< 0.01
	Genetic relatedness	0.17	0.07	5.78	2.41	0.02
	Stability (stable)	0.03	0.13	0.07	0.26	0.79
	Group size	0.11	0.04	8.01	2.83	< 0.01
	Stability (stable): Sex (same-sex)	0.12	0.05	4.52	2.11	0.03
	Stability (stable): Rank difference	-0.19	0.07	8.38	-2.90	< 0.01
Proximity (< 3 BL)	(Intercept)	-0.56	0.64	0.76	-0.87	0.38
	Sex (same-sex)	0.87	0.10	75.13	8.67	< 0.001
	Rank difference	-0.32	0.20	2.72	-1.65	0.10
	Familiarity (familiar)	0.37	0.08	19.16	4.38	< 0.001
	Genetic relatedness	-0.70	0.26	7.53	-2.74	< 0.01
	Stability (stable)	-0.30	0.30	0.91	-0.95	0.34
	Group size	-0.29	0.10	4.39	-2.09	0.04
	Stability (stable): Rank difference	0.60	0.24	5.93	2.44	0.01
Grooming frequency	(Intercept)	-0.75	0.88	0.74	-0.86	0.39
	Sex (same-sex)	0.15	0.25	0.35	0.59	0.55
	Rank difference	-0.34	0.41	0.67	-0.82	0.41
	Familiarity (familiar)	0.96	0.33	8.34	2.88	< 0.01
	Genetic relatedness	1.47	0.92	2.52	1.59	0.11
	Stability (stable)	-0.52	0.38	1.86	-1.37	0.17
	Group size	-0.39	0.13	9.13	-3.02	< 0.01

Table 3. Summary of statistics for three separate generalized linear mixed models (GLMMs) for investigating the effects of social predictors (fixed effects) on inter-individual distance, proximity (< 3 BL) and grooming frequency. GLMMs control for the ID of the two horses within the dyad, group ID and the date of observation. Significance is set at $P < 0.05$.