The multilevel society of proboscis monkeys with a possible patrilineal basis

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Abstract

Multilevel societies (MLS), which have two or more levels of organization, are among the most complex primate social systems. MLS has only been seen in some primates, including humans. The aim of this study was to identify MLS in proboscis monkeys in Sabah, Malaysia, through direct observation (>32 months) and genetic analysis, based on feces collected from ~200 individuals, to estimate the degree of kinship. The results revealed that the social system of the proboscis monkey exhibited a form of MLS, with several core reproductive units and the bachelor group woven together into a higher-level band. Genetic analysis revealed that the females migrated randomly over short and long distances; however, the males tended to migrate relatively shorter distances than females. Combined with the results of direct observations, we concluded that proboscis monkeys form an MLS with a double or triple structure and patrilineal basis. The results of this study may have implications for the evolution of human societies, as to why patrilineal MLS, an important step in the evolutionary path of human societies, emerged in phylogenetically distant primates, such as the proboscis monkey.

Keywords: human evolution; *Nasalis larvatus*; primates; *Rhinopithecus roxellana*; social structure

Introduction

Primate multilevel societies (MLS) are a form of social organization where the joint activities of a set of core social units (foraging, resting, and traveling) scale-up to generate higher societal levels, such as bands (Grueter *et al.* 2020). Units do not have territorial boundaries, but ranges, which completely overlap with those of other units. Interactions with other units are characterized by a lack of persistent hostility, with units generally tolerating each other's presence. Although the units are part of a larger collective (e.g., a band), they can temporarily bud off from the band (e.g., for foraging). This form of fission–fusion where the integrity of the core unit is maintained, is distinct from the more atomistic pattern seen in single-tiered societies, such as those of chimps and spider monkeys, where a group (community) divides into smaller sub-groups (parties) of variable size or composition and each community has its own territory or home range (Grueter *et al.* 2012a; Rodseth *et al.* 1991).

A prime example of a species where MLS are almost universally present is humans (Dyble *et al.* 2016; Grueter *et al.* 2012a; Rodseth *et al.* 1991). Human MLS are based on family groups that are integrated into a network of increasingly larger social tiers, such as bands and tribes (Layton & O'Hara 2010). The MLS of nonhuman primates are not as complex as human MLS; however, several tiers of social stratification can be found in a limited number of species (Grueter *et al.* 2012b). MLS have evolved in African papionin species, such as geladas (*Theropithecus gelada*) and hamadryas baboons (*Papio hamadryas*) (Dunbar & Dunbar 1975; Kummer 1968; Swedell 2011; Swedell & Plummer 2012), and in Asian colobine species, in particular, the odd-nosed group, including snub-nosed (*Rhinopithecus* spp.) and proboscis monkeys (*Nasalis larvatus*) (Grueter *et al.* 2022). The minimum reproductive unit in the MLS of most nonhuman primates is the one-male–multifemale group, but MLS that are not based on such groups, i.e., multimale–multifemale groups, have recently been reported in Guinea baboons (*Papio papio*) (Patzelt *et al.* 2014) and Rwenzori colobus (*Colobus angolensis ruwenzorii*) (Miller *et al.*

2020; Stead & Teichroeb 2019).

This nested arrangement of grouping tiers allows individuals to be concurrently associated with multiple grouping levels and harnesses the adaptive benefits that each level entails (Grueter *et al.* 2017). For example, the band level allows core units to 'recruit' extra-unit males for collective defense when needed (Xiang *et al.* 2013) and the core unit offers a safe haven for individuals to exchange social services, such as grooming or allocare (e.g. Dunbar 1979; Yu *et al.* 2013). When molecular fission–fusion is superimposed on a multilevel organization, the system becomes even more flexible, as core units also have the option to separate from the higher-level group when the availability and distribution of food resources demands it (Schreier & Swedell 2012).

Other taxa presenting MLS are generally absent in the immediate phylogenetic neighborhood of taxa presenting MLS, though evidence is inconclusive in some cases (Grueter & Wilson 2021). Thus, great apes are not ideal models to reconstruct MLS in humans (but see Morrison *et al.* 2019). Consequently, studies on primates that are phylogenetically distantly related but exhibit multilevel structures are important for elucidating the evolutionary paths leading to MLS in humans (Grueter *et al.* 2012a).

Proboscis monkeys are endemic to the island of Borneo. This species is a large, sexually dimorphic arboreal colobine that typically inhabits mangrove forests, peat swamps, and riverine forests (Grueter *et al.* 2022). The basic components of their society, i.e., the minimum reproductive units (core units), are one-male–multifemale units (OMUs) which assemble with each other in riverside trees (Bennett & Sebastian 1988; Boonratana 2002; Yeager 1991), with varying degrees of aggregation depending on seasonal fluctuations in food abundance or predation pressure (Matsuda *et al.* 2010a). Several studies suggest that there is a multilayered social organization to this association of OMUs along the river (Bennett & Sebastian 1988; Boonratana 2002; Yeager 1991), although one study suggests that ecological factors, such as food availability and predation pressure, would be sufficient to explain the cohesion of OMUs without assuming a higher order structure of this type (Matsuda *et al.* 2010a). In addition to OMUs, all-male or bachelor groups (AMGs) are also found, consisting mostly of immature males who are often accompanied by adult males (Matsuda *et al.* 2020a; Murai 2004). Although the AMG is a source for future OMU males (Murai 2004), little is known about their behavioral ecology. In addition, no studies have examined the social structure of proboscis monkeys from a genetic basis.

The aim of this study was to determine the social structure of proboscis monkeys by direct observation and genetic analysis. The six-month study of Yeager was based on the identification of multiple groups (OMUs and AMGs) and examining the interactions between them (Yeager 1991); however, we observed long-term interactions between the groups for >32 months. Given that proboscis monkeys form MLS, certain OMUs are expected to frequently maintain close proximity on their sleeping trees along the river. To gain a comprehensive picture of their complex society, we analyzed the degree of kinship between individuals within a population of proboscis monkeys through fecal DNA, albeit at a different time of the year from the time of direct observation. In proboscis monkeys, both males and females disperse from their natal OMUs to other OMUs or AMGs before they reach maturity. However, their social networks, based on grooming interactions, are decidedly female-centered (Matsuda *et al.* 2012a; Yeager 1990). We assume that the emergence of societies in which females are central to social interactions would be based on a high degree of kinship between females within OMUs. Therefore, we hypothesize that although both sexes disperse between groups, females would tend to disperse to more proximal OMUs than males (Guo *et al.* 2015; Snyder-Mackler *et al.* 2014, but see Fang *et al.* 2022). We also hypothesize that males disperse over longer distances than females, whose dispersal distances are shorter. Lastly, we discuss the similarities and differences between the findings from this study and those from studies on closely related snub-nosed monkeys, with better studied MLS (Grueter 2022), and consider what our results could contribute to our understanding of the social evolution of humans.

Methods

Study site and animals

We conducted the study in a riverine forest along the Menanggul River, a tributary of the Kinabatangan River, Sabah, Malaysia (118°30′ E, 5°30′ N). The mean minimum and maximum daily temperatures were approximately 24°C and 30°C, respectively, and the mean annual precipitation at the site was 2,474 mm (Matsuda *et al.* 2019). The river level varied by approximately 1 m daily. The average river level rose >3 m during seasonal flooding. The riverine forest was inhabited by eight diurnal primate species, including our study subject, the proboscis monkey. Proboscis monkeys under observation were well habituated to observers in boats, because this area is one of the main tourist attractions in the region, with many boats and tourists visiting the Menanggul River almost on a daily basis during the last decade.

Behavioral observation in 1991–2001

Along the Menanggul River, using a GPS unit, location points were collected at intervals of 50 m over a transect stretching from the river mouth of the Menanggul River up to a point 6 km upstream. Proboscis monkeys typically return at night to the riverbank to sleep in our study site (Matsuda *et al.* 2010b). In this study, the core observation area was set up from the river mouth up to 4 km upstream, where boat-based observations were performed in the early morning (06:00–09:00) and/or late afternoon (15:00–18:00). However, if we could not find groups of proboscis monkeys in the area for up to 4 km, we extended the survey to the upstream area of up to 6 km.

We conducted the behavioral observations from February 1999 to October 2001, and in May and June 2002 (560 d). By late June 1999, eight OMUs and one AMG was identified by distinguishing all adult males and a few adult females based on their physiognomic external features, such as scars and nose shapes. The number of OMUs observed in the study area was consistently eight (Koda *et al.* 2018). The replacement of the dominant males, named Ba, Wa, and Bu, was observed on three occasions during the study period in August 2000, November 2000, and October 2001, respectively (Murai *et al.* 2007). In each case, the replacing males were derived from the AMGs. We refer to OMUs before the male replacement occurred as "senior" OMUs (Ts, Be, Po, Bo, Yo, De, Pu, and Ki) and OMGs after the male replacement as "junior" OMUs (BoWa, PoBu, and KiBa) for the sake of convenience. Throughout the study period, the mean number of individuals comprising OMUs was 18, ranging from 8 to 34, and AMGs consisted of approximately 30 males (Supplementary Table 1). The names of the OMUs and AMGs and the locations of their sleeping sites were recorded whenever proboscis monkeys were found along the river in the late afternoon.

Genetic analysis

DNA sampling in 2015–2016

From July 2015 to April 2016, we collected fecal samples from proboscis monkeys in the study area for genetic analyses. A boat survey was conducted in the late afternoon to detect the group locations and record the GPS coordinates of their sleeping sites: early morning the next day, when the monkeys were still asleep; the sites were revisited while we waited in the boat until the monkeys moved inside the forest. As proboscis monkeys often defecated shortly before moving into the forest, fresh feces were collected by carefully exploring the ground nearby their sleeping trees after they had left them. We rubbed the surface of the fecal pellets with cotton swabs, and dipped the swabs in 2 mL tubes containing 1 mL lysis buffer, consisting of 0.5% SDS, 100 mM EDTA, 100 mM Tris-HCl, and 10 mM NaCl (Longmire *et al.* 1997). The swabs were discarded and the tubes were stored at room temperature for ~400 d after collection until they were delivered to the laboratory, where they were kept at −80°C until DNA extraction. The DNA of 307 samples was extracted with QIAamp DNA Stool Mini Kit (Qiagen) using TE (1 M Tris-

Cl, 0.05 M EDTA; pH 8.0) as elution buffer.

Microsatellite screening and sex identification

Twenty-four markers (23 polymorphic microsatellite loci and 1 fragment of the DEAD-box gene) were amplified for genetic analyses. The DEAD-box gene was used to identify sex (Inoue *et al.* 2016). The microsatellite loci were selected based on the method of Inoue *et al.* (2016) and Salgado-Lynn *et al.* (2010a), with four PCR multiplex reactions optimized for this study (Supplementary Table 1).

PCR was performed using the Applied Biosystems Veriti 96 Well Thermal Cycler PCR machine. All PCR amplifications were performed in 15 μL reactions, containing 1 × Master Mix (Qiagen Multiplex Kit), 0.4 μg/μL of BSA, 0.2 μM of each primer used for the multiplex combinations, and 2 μL of 10–100 ng of template DNA. After an initial incubation at 95°C for 15 min, PCR amplification was performed for 40 cycles, consisting of denaturation at 94°C for 45 s, annealing at 60°C or 62°C for 90 s, extension at 72°C for 90 s, and a final extension at 72°C for 10 min. Then, 2% agarose gels were used to visualize band quality and verify target band size. The PCR products were sent to First BASE Laboratories (Kuala Lumpur, Malaysia) for fragment analysis. The results of fragment analysis were scored using the software GeneMarker version 2.6.3 and corrected by eye.

We used 20 samples for a pilot study to determine the number of positive PCR repetitions needed to obtain a reliable genotype. A consensus threshold (100 simulations; the range of repetitions was from two to several) was produced in GEMINI v.1.4.1 (Valière *et al.*, 2002) in the "Consensus Threshold Test" module. The consensus threshold was used in a "PCR Repetition Test," also in GEMINI (1,000 simulations; the range of repetition was two to 10), which showed that three positive repetitions for each multiplex were sufficient to achieve a reliable genotype, with a maximum of five positive repetitions to clarify ambiguities. However, if there were no PCR products after three PCR rounds in two or more multiplexes, the sample was not included in the analysis.

Of the 307 samples from which DNA extraction was performed, 267 fecal samples were genotyped, and only 197 were included in relatedness analysis (<10% missing data). GenAlEx 6.51b2 (Peakall and Smouse, 2006, 2012) was used to calculate heterozygosity, frequency-based statistics, and polymorphism (see Table 1). A Hardy–Weinberg Equilibrium (HWE) exact test was performed separately for males ($N = 64$) and females ($N = 133$) in Genepop 4.7.5 (Raymond & Rousset 1995; Rousset 2008) using default Markov chain parameter values. Neither group was in HWE ($p < 0.001$).

Data analysis

Degree of association between groups

Based on the distance between the edges of the sleeping site location of each OMU or AMG along the river, they were considered to be alone or in association. In association was defined as 100 m or less between the edges of the sleeping site of the relevant OMU or AMG. The rationale for this approach follows previous studies where OMUs of few individuals or OMUs with identified or unidentified individuals were considered members of an OMU if they were observed to be within a distance of 100 m of an OMU (Kawabe & Mano 1972; Kern 1964; Macdonald 1982; Salter *et al.* 1985). This distance was recommended as being operationally suitable by Yeager (1992). However, the frequency with which the relevant groups had sleeping sites within 100 m from each other is not an appropriate index of the degree of their association as the frequency of appearance of each group along the river varied. Thus, to assess intergroup associations, we calculated the percentage of days when the relevant groups had sleeping sites within 100 m from each other, based on the number of days when the sleeping sites of relevant groups were detected along the river.

We generated weighted social networks between the groups based on the degree of association calculated using the R package 'igraph' (Csardi & Nepusz 2006). In this study, two types of social network diagrams were drawn including all study groups, i.e., 8 OMGs and 1 AMG, before and after the first observed replacement of the dominant male in the OMG. Additionally, we used cluster analysis (Quinn & Keough 2002) to assess similarity relationships for the associations among study groups based on the same dataset using the social networks. The degree of association among groups was clustered using Euclidean distance and Ward's linkage method (Quinn & Keough 2002). The quality of a clustering was validated by the analysis of silhouette values (Rousseeuw 1987), which vary from −1 to 1 and represent the tightness of the data points within a cluster and the separation between different clusters in a given model. The silhouette value $S(x_i)$ for a single data point x_i is computed as

$$S\ (x_i)\ =\frac{b\ (x_i)\ -\ a\ (x_i)}{max\ (a(x_i), b\ (x_i))}$$

where $a\ (x_i)$ is the average Euclidean distance of x_i to all points in the same cluster, and $b\ (x_i)$ is the minimum average Euclidean distance of x_i to all other clusters in which x_i is not a member. Thus, low values of $a\ (x_i)$ indicate that x_i is representative of the cluster, whereas high values of $b\ (x_i)$ indicate that x_i is much different from the other clusters. Higher values denote higher clustering quality, whereas negative values denote that the data point should otherwise belong to a different cluster. By computing,

$$S_k\ =\frac{1}{n_k}\sum\nolimits_{x_i \in C_k} S\ (x_i)$$

as the average of $S(x_i)$ over all n_k points in cluster C_k, one obtains an indicator of how well separated the cluster is from all others. An indicator for the complete clustering is obtained by averaging S_k over all K deduces both $a(x_i)$ and $b(x_i)$ simultaneously, and thus, a larger number of clusters does not necessarily improve the silhouette score. This property makes the silhouette a better indicator for cluster quality than, for example, the log-likelihood of data, because the latter merely increase with a larger K.

Relatedness and Fst

Relatedness between all pairs of individuals was estimated using the Moment Estimate of Relatedness method of Wang (2002) and mean total relatedness (R) and its standard deviation after 10,000 bootstrap replicates, used to simulate allele frequencies. Female and male relatedness was examined using the Kolmogorov–Smirnov test (KS-test) in R. To compare relatedness, first, we used pairwise comparisons of the individuals who presented a relatedness of 0.001 or higher to exclude the bulk of the relatedness values that were zero or negative, because these indicate individuals that are not related, e.g., because they derive from different populations and share no genetic variation. Second, we compared the relatedness ≥0.25 between females with that between males to determine the differences between females and males of higher kinship levels.

The effect of spatial autocorrelation was measured to determine the effect of geographical distance between individuals and the relatedness they showed for females and males separately. For this, we used the geographic distance between individuals measured with respect to the mouth of the river and R values between them with negative values converted to zero (i.e., no kinship). Based on these data, we tested whether there was a correlation between geographical distance and genetic distance (isolation by distance) using the Kendall rank correlation coefficient.

Based on the location points along the river (distance from the river mouth) where the fecal samples were collected, we divided the locations into distance bins that reflected samples separated from each other by i) 0–500, ii) 501–1000, iii) 1501–2000, iv) 2001–2500, v) 2501–3000, vi) 3001–3500, and vii) 3501–4000 m. Notably, we excluded zone 1001–1500 m, because we collected only one fecal sample in that section. Within those sampling zones, we estimated pairwise divergence F_{ST} of Wright (1951) with the method of Weir and Cockerham (1984) between all pairs of zones and used Mantel tests to determine the relationship of the pairwise F_{ST} matrix and the matrix of geographical distance between pairs of zones, e.g., two adjacent zones were separated by

500 m, whereas the most distant zones were separated by 4000 m. The *p*-value threshold for the Mantel test was corrected with the FDR method of Benjamini and Yekutieli (2001).

Results

Direct observation of social structure in 1991–2001

The total number of observations per group is provided in Supplementary Table 2. Groups differed in the number of observations, ranging from 12 (PoBu) to 366 (Po). The weighted graph, based on the degree of association of each group pair, is shown in Figure 1a. Based on the evaluation using silhouette values, hierarchical cluster analysis indicated two major clusters, i.e., band A, including the OMUs, Ts, Be, Po, and Bo ($S_k = 0.236$); and band B, including the OMUs, Yo, De, Pu, and Ki, and the AMG ($S_k = 0.273$; Fig. 1b). Two junior OMUs (BoWa and PoBu) kept belonging to band A ($S_k = 0.164$) even after those groups were replaced by young males (Wa and Bu) provided from the AMG (Fig. 1c). However, another junior OMU (KiBa), moved to band A from band B ($S_k = 0.293$) after the group of Ki was replaced by Ba. The AMG flexibly changed the band while replacements occurred (Fig. 1c).

The two bands in the study site were likely to segregate to areas in which they slept along the Menanggul River, although these widely overlapped. The upstream area of the Menanggul River was predominantly occupied by band A, as shown by a mean distance of sleeping sites from the river mouth of 923 m, ranging from 0 m to 2000 m (Fig. 2). By contrast, band B mostly occupied the downstream area, with the mean sleeping site distance from the river mouth of 2,042 m, ranging between 400 and 5,800 m. The home range of the AMG spread over the Menanggul River, with a mean sleeping site distance from the mouth of the Menanggul of 2,837 m and a wider range from 0 m to 6,000 m.

Genetic analysis of social structure in 2015–2016

Overall relatedness pattern

Of 307 samples from which DNA extraction was performed, a total of 267 fecal samples were genotyped, and only 197 were included in relatedness analysis (<10% missing data). The total number of relatedness pairwise comparisons measured among the 197 samples was 35,511, of which 27,777 (78%) were negative numbers, thus indicating that those pairs of samples are not related to each other as they share no genetic variation (e.g., the adults are migrants). On the remaining data (positive values), we found that there were not identical twins. A total of 187 parent/offspring or full sibling relationships were identified, and ~1207 relationships could be considered cousins or half-siblings (Figure 3).

Female vs. male relatedness

Comparisons of relatedness between males and females in pairs showing a mean relatedness >0.001 did not show significant differences (KS-test *p*-value = 0.4048). However, performing the same test only on those pairwise comparisons with a relatedness of ≥0.25 resulted in significant differences between the two sexes (KS-test *p*-value = 0.0097), with the males showing higher relatedness values (0.40759 ± 0.1485) than the females (0.3805 ± 0.137).

Relatedness based spatial autocorrelation

A significant negative correlation between geographic distance and genetic distance was found for both sexes, with females (N = 133) showing a negative Kendall's $\tau = -0.066$ (*p*-value = 1.153e-15) and males (N = 64) showing a slightly less negative Kendall's $\tau = -0.053$ (*p*-value = 0.00153; Figure 4). Overall, as geographic distance increased, the degree of relatedness decreased, indicating that the more distant the two individuals were, the lower their relatedness.

Given that our analysis counted the pairwise relatedness of each sample as a single point, the statistical power to detect a relationship between degree of relatedness and geographical distance was potentially weak. To increase the statistical power of the data, we subdivided the entire set of samples in zones (i.e., with multiple pairwise comparisons per zone) and tested for a relationship between the mean relatedness between zones and the corresponding geographic distance. For each sex, a significant reduction was observed in relatedness as geographical distance increased (female Pearson's $r = -0.906$, p-value = 0.0048; male Pearson's $r = -0.947$, p-value = 0.001182), with approximately 82% of the difference in relatedness explained by distance for females and 89% for males (Figure 5).

Pairwise differentiation along the river and isolation by distance

The relationship between geographical distance and degree of differentiation (F_{ST}) was examined after dividing the samples of each individual into zones based on the location from which their fecal samples were collected. Significant differences were found in the degree of F_{ST} in the pairwise comparisons of 5 out of 21 zones for females and 4 out of 21 zones for males (Table 2). Males tended to exhibit significant differentiation with individuals living in the uppermost zone of the river (3501–4000 m) from those living near the river mouth, which is indicated by an increase in genetic differentiation with an increase in geographical space between them (Mantel test squared correlation coefficient $r^2 = 0.603$, p-value = 0.0055), and thus, approximately 60% of the difference in allele frequency between males can be explained by the geographical distance between the sampling points. However, females showed no such consistent trend, and no significant differentiation was found despite geographical distance (Mantel test squared correlation coefficient $r^2 = 0.022$, p-value = 0.67302).

Discussion

The findings of this study revealed that the social system of the proboscis monkey takes the form of an MLS, with OMUs (core reproductive units) and the AMG (bachelor group) woven together into a higher-level band. Several studies have reported that OMUs of proboscis monkeys are not territorial and sleep in close proximity in the trees along the riverside where they spend the night (Bennett & Sebastian 1988; Boonratana 2002). In particular, the study by Yeager (1991), in which multiple groups were identified and their relationships examined over six months, provides the most detailed analysis of the social organization of proboscis monkeys. The results of Yeager are generally in accordance with our results: two bands consisting of multiple OMUs and AMGs were detected, and the two bands were loosely segregated upstream and downstream of the river, with a certain degree of overlap in their distribution ranges.

Although this study confirmed that the proboscis monkey forms MLS, a previous study conducted by us at the same site (Matsuda *et al.* 2010a) was ambiguous in this regard. That study showed that changes in local density of OMUs along the river can be predicted by spatial heterogeneity along the river, i.e., locations with a narrower river width are advantageous for predator avoidance, because animals can escape by leaping to the other side of the river when attacked by a predator, and temporal changes in diet, i.e., better foraging locations where clumped food patches are abundant. In other words, the local density of OMUs may increase where better sleeping conditions are available, suggesting that associations among units are induced by habitat features and are not necessarily the outcome of social attraction. However, this previous study focused on only one identified focal OMU, whereas other OMUs that stayed around that focal OMU were not identified and treated as local density. Hence, the aspect of whether a particular affinity among specific OMUs existed during the period of increased local density was ignored. Integrating the results of our previous study and this study, it appears that the proboscis monkeys form MLS with specific OMUs at their core, and that the degree of association among units varies seasonally, depending on food abundance and predation pressure. Indeed, the association between identified OMUs of proboscis monkeys at other study sites have been reported to be seasonal, although no ecological explanations for such seasonality were provided (Yeager 1991). A detailed review has shown that ecological conditions, such as food abundance, are merely permissive and do not seem to drive the nested nature of the MLS of Asian colobines (Grueter & van Schaik 2009). However, it is premature to discard the impact of ecological factors, such as resource abundance and predation pressure, on the evolution of MLS in proboscis monkeys (see also Grueter 2022).

In our longer-term study of 27 months, we observed three occasions of adult male replacement in the OMUs, which offered new insights into the social system of the proboscis monkey. We found that, after the replacement of the dominant male, an OMU would occasionally switch band affiliation. Changes in band affiliation have also been documented for the AMG. Our observational protocol did not allow us to explore the reasons behind switches in band affiliation. These could include avoidance of social disadvantages, e.g., a low unit dominance rank. Understanding the motivations for changes in band affiliation requires further detailed individual-level observations of both OMUs and AMG.

Although several genetic studies have been performed on proboscis monkeys, e.g., population genetics focusing on mitochondrial control region (Munshi-South & Bernard 2011), development of polymorphic microsatellite loci (Inoue *et al.* 2016; Salgado-Lynn *et al.* 2010), and their application to zoo studies (Ogata & Seino 2014), this study was the first to explore their social structure genetically by estimating relatedness in a large number of individuals (N > 190), in the wild. The negative correlation between genetic and geographic distances is comparable to that reported for many other primates (e.g. Hagell *et al.* 2013; Mbora & McPeek 2014; Oklander *et al.* 2017). By contrast, the significantly higher degree of relatedness between males than females in the population at the study site was rather unexpected in the proboscis monkey, for which a more female-centered social interaction network has been observed (Matsuda *et al.* 2012a; Matsuda *et* *al.* 2012b; Yeager 1990). Thus, social bonds between females may be driven by factors other than genetics. A notable feature of social interactions in colobine monkeys, including proboscis monkeys, involves non-mothers handling infants, i.e., allomothering behavior (Davies & Oates 1994; Matsuda *et al.* 2022; Matsuda *et al.* 2012b), although sometimes handlers hurt or abuse the infant and do not provide genuine care (McKenna 1979). Social bonds between females may be enhanced through such allomothering behaviors (Matsuda *et al.* 2015; Zhang *et al.* 2012), which can have various functional benefits, e.g., increased foraging efficiency for mothers and parental training of nulliparous females (Maestripieri 1994). Therefore strong ties between females within groups may develop even in the absence of close relatedness (McKenna 1979).

F_{ST}-based analyses indicated that although females randomly migrated short or long distances, males were more likely to migrate relatively shorter distances than females and rarely migrated long distances, such as from the mouth of the river to >4,000 m upstream. This may simply indicate that in the two different bands, males disperse more proximally within the band to which they belong, whereas females can disperse proximally or distally within and out of the band. However, when considering the results of the direct observations conducted in different periods from genetic research, it is more likely that males disperse more distally than females (Figure 2), which is seemingly contradictory to the results of F_{ST} analysis. Assuming that a larger community structure encompassing bands A and B, which might be termed a “troop”, exists in the proboscis monkey society (Figure 6) may explain why F_{st} was significantly different between males in the upper and lower streams. Males may disperse within a band as well as between bands, whereas females may be able to disperse beyond the troop. Because the MLS of golden snub-nosed monkeys (*Rhinopithecus roxellana*), a phylogenetically close relative of proboscis monkey, has been proposed to form grouping levels beyond the two-tier structure of OMUs and bands (Figure 6: Qi *et al.* 2014; Qi *et al.* 2017), the possibility of a three-tiered social organization in proboscis monkey cannot be ruled out. Extensive genetic sampling and parallel observations of the behaviors of multiple identified units are required to verify this assumption.

The MLS of proboscis monkeys are comparable to that of snub-nosed monkeys, especially in terms of the flexible dispersal patterns seen in both sexes (Qi *et al.* 2009; Zhang *et al.* 2012). However, there is a subtle difference in that in snub-nosed monkeys, each breeding band composed of OMUs is accompanied by AMUs to form a herd (Qi *et al.* 2020; Qi *et al.* 2014), whereas in proboscis monkeys, the AMG covers multiple bands composed of OMUs. This inference is based on both genetic data and direct observations (Figure 6). As discussed above, results from the genetic data alone suggest that proboscis monkeys exhibit an MLS similar to that of snub-nosed monkeys. Although the lack of analysis of individual-level behavior is a limitation of this study, the more constrained dispersal patterns in males than those in females (which did not seem to migrate beyond the troop) may be a difference between the social systems of proboscis and snub-nosed monkeys. Accordingly, it would be unique within a band or herd that a patrilineal genetic basis is formed in the proboscis monkey. Assuming patrilineal MLS in proboscis monkeys, the following two observations are possible explanations: 1) male–male fighting causing serious injury is rarely observed in proboscis monkeys, although sexual competition between males is intense, as evidenced by their large noses and body sizes (Koda *et al.* 2018; Matsuda *et al.* 2020b), and 2) infanticide has not been observed in natural populations. Invoking inclusive fitness theory, if males are related to each other within a band or herd, the adult males of the OMUs are expected to tolerate each other and avoid excessive fighting over females, and likewise, the males of AMGs are expected to avoid taking over the group through serious physical challenges to the adult males of the OMUs. This aspect of the social system of proboscis monkey bears resemblance to the society of hamadryas baboons where males of the same clan (the tier above the OMU in their MLS) are philopatric, patrilineally related, and spatially associated and frequently engage in social interactions and show mutual respect of female 'ownership' (Abegglen 1984;

Kummer *et al.* 1974; Schreier & Swedell 2009; Städele *et al.* 2014). However, this scenario differs from that the non-patrilineal based MLS of *R. roxellana*, where there is even collective action involving OMU males (Xiang *et al.* 2013).

Although infanticide has been reported in proboscis monkeys under provisioned conditions with a high population density (Agoramoorthy & Hsu 2005), infanticide has not been reported under natural conditions. Females with infants have also been reported to transfer to other OMUs, but no infanticide or attacks by dominant males have been observed in these cases (Bennett & Sebastian 1988; Matsuda *et al.* 2012a). After the takeover of an OMU by a male from an AMG, the risk of infanticide will be reduced if the usurping male is related to the ousted male. Hence, the mechanism underlying the lack of infanticide in proboscis monkeys may be different from that of the snub-nosed monkey, where infanticide is thought to be constrained by paternity uncertainty resulting from extra-unit mating (Qi *et al.* 2020). Because extra-unit mating has not been observed in proboscis monkeys, it is unlikely that a similar scenario would act as an additional or alternative deterrent to infanticide. Understanding why an MLS with a patrilineal basis, a key step in the evolutionary history of human society (Chapais 2008), emerged in a phylogenetically distantly related primate species, such as the proboscis monkey, may provide insightful clues about human social evolution.

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Table 1 Summary statistics of genetic variations between male and female proboscis monkey populations in the Menanggul River. N: sample size, Na: number of alleles, Nea: number of effective alleles, Ho: observed heterozygosity, He: expected heterozygosity, F: inbreeding coefficient, HWE: Hardy–Weinberg Equilibrium, *: significant, NS: not significant, SE: standard error for the population mean and NA: not applicable.

Males							
Locus	**N**	**Na**	**Nea**	**Ho**	**He**	**F**	**HWE**
NlE10	59	7.000	2.437	0.373	0.590	0.368	*
NlP2C12	64	5.000	2.167	0.250	0.538	0.536	*
NlP3B6	63	6.000	3.202	0.587	0.688	0.146	*
NlP1C5	60	4.000	1.860	0.333	0.462	0.279	*
NlP2B8	60	5.000	3.668	0.517	0.727	0.290	*
DEADBOX	64	2.000	1.969	0.844	0.492	-0.714	*
NlP2D6	61	8.000	3.474	0.492	0.712	0.309	*
NlP3C11	62	4.000	2.133	0.210	0.531	0.605	*
NlP2B9	54	7.000	4.893	0.463	0.796	0.418	*
NlP3H5	60	4.000	2.131	0.467	0.531	0.121	NS
NlP3B4	62	3.000	1.752	0.129	0.429	0.699	*
NlP4B2	58	3.000	1.496	0.017	0.332	0.948	*
NlD10	60	1.000	1.000	0.000	0.000	NA	NA
NIP2F7	63	3.000	2.881	0.810	0.653	-0.240	NS
NlP2F3	60	3.000	2.799	0.250	0.643	0.611	*
NIP3G2	61	10.000	5.066	0.574	0.803	0.285	*
NIP4B1	64	2.000	1.133	0.063	0.117	0.467	*
NIP4E10	62	5.000	4.406	0.661	0.773	0.145	*
NIP4C11	62	9.000	5.559	0.726	0.820	0.115	*
NIP1A6	64	2.000	1.771	0.422	0.435	0.031	Ns
NIP2C5	64	4.000	2.787	0.656	0.641	-0.023	NS
NIP3B2	64	5.000	2.260	0.484	0.557	0.131	NS
NIP3E8	60	5.000	3.952	0.650	0.747	0.130	NS
Mean	61.348	4.652	2.817	0.434	0.566	0.257	*
SE	0.509	0.485	0.265	0.051	0.044	0.072	NA
Females							
Locus	**N**	**Na**	**Ne**	**Ho**	**He**	**F**	**HWE**
NlE10	130	6.000	3.619	0.262	0.724	0.639	*
NlP2C12	129	6.000	2.413	0.233	0.586	0.603	*
NlP3B6	128	7.000	4.118	0.477	0.757	0.371	*
NlP1C5	122	6.000	1.957	0.385	0.489	0.212	*
NlP2B8	127	5.000	2.868	0.299	0.651	0.541	*
DEADBOX	133	2.000	1.062	0.000	0.058	1.000	*
NlP2D6	126	6.000	2.782	0.421	0.640	0.343	*
NlP3C11	125	4.000	1.652	0.240	0.395	0.392	*
NlP2B9	100	7.000	4.311	0.270	0.768	0.648	*
NlP3H5	128	4.000	1.971	0.320	0.493	0.350	*

NIP3B4	132	4.000	1.272	0.114	0.214	0.468	*
NIP4B2	126	2.000	1.083	0.000	0.076	1.000	*
NID10	125	1.000	1.000	0.000	0.000	NA	NA
NIP2F7	127	3.000	2.674	0.693	0.626	-0.107	*
NIP2F3	127	7.000	2.790	0.181	0.642	0.718	*
NIP3G2	119	10.000	4.313	0.521	0.768	0.322	*
NIP4B1	123	2.000	1.185	0.057	0.156	0.636	*
NIP4E10	124	8.000	4.630	0.508	0.784	0.352	*
NIP4C11	120	9.000	5.791	0.650	0.827	0.214	*
NIP1A6	133	2.000	1.975	0.361	0.494	0.269	*
NIP2C5	132	5.000	3.329	0.644	0.700	0.080	*
NIP3B2	132	5.000	2.549	0.447	0.608	0.265	*
NIP3E8	127	5.000	3.737	0.504	0.732	0.312	*
Mean	125.870	5.043	2.743	0.330	0.530	0.438	*
SE	1.430	0.497	0.275	0.044	0.054	0.056	NA
Males							
Locus	**N**	**Na**	**Nea**	**Ho**	**He**	**F**	**HWE**
NIE10	59	7.000	2.437	0.373	0.590	0.368	*
NIP2C12	64	5.000	2.167	0.250	0.538	0.536	*
NIP3B6	63	6.000	3.202	0.587	0.688	0.146	*
NIP1C5	60	4.000	1.860	0.333	0.462	0.279	*
NIP2B8	60	5.000	3.668	0.517	0.727	0.290	*
DEADBOX	64	2.000	1.969	0.844	0.492	-0.714	*
NIP2D6	61	8.000	3.474	0.492	0.712	0.309	*
NIP3C11	62	4.000	2.133	0.210	0.531	0.605	*
NIP2B9	54	7.000	4.893	0.463	0.796	0.418	*
NIP3H5	60	4.000	2.131	0.467	0.531	0.121	NS
NIP3B4	62	3.000	1.752	0.129	0.429	0.699	*
NIP4B2	58	3.000	1.496	0.017	0.332	0.948	*
NID10	60	1.000	1.000	0.000	0.000	NA	NA
NIP2F7	63	3.000	2.881	0.810	0.653	-0.240	NS
NIP2F3	60	3.000	2.799	0.250	0.643	0.611	*
NIP3G2	61	10.000	5.066	0.574	0.803	0.285	*
NIP4B1	64	2.000	1.133	0.063	0.117	0.467	*
NIP4E10	62	5.000	4.406	0.661	0.773	0.145	*
NIP4C11	62	9.000	5.559	0.726	0.820	0.115	*
NIP1A6	64	2.000	1.771	0.422	0.435	0.031	Ns
NIP2C5	64	4.000	2.787	0.656	0.641	-0.023	NS
NIP3B2	64	5.000	2.260	0.484	0.557	0.131	NS
NIP3E8	60	5.000	3.952	0.650	0.747	0.130	NS
Mean	61.348	4.652	2.817	0.434	0.566	0.257	*
SE	0.509	0.485	0.265	0.051	0.044	0.072	NA
Females							
Locus	**N**	**Na**	**Ne**	**Ho**	**He**	**F**	**HWE**
NIE10	130	6.000	3.619	0.262	0.724	0.639	*

NlP2C12	129	6.000	2.413	0.233	0.586	0.603	*
NlP3B6	128	7.000	4.118	0.477	0.757	0.371	*
NlP1C5	122	6.000	1.957	0.385	0.489	0.212	*
NlP2B8	127	5.000	2.868	0.299	0.651	0.541	*
DEADBOX	133	2.000	1.062	0.000	0.058	1.000	*
NlP2D6	126	6.000	2.782	0.421	0.640	0.343	*
NlP3C11	125	4.000	1.652	0.240	0.395	0.392	*
NlP2B9	100	7.000	4.311	0.270	0.768	0.648	*
NlP3H5	128	4.000	1.971	0.320	0.493	0.350	*
NlP3B4	132	4.000	1.272	0.114	0.214	0.468	*
NlP4B2	126	2.000	1.083	0.000	0.076	1.000	*
NlD10	125	1.000	1.000	0.000	0.000	NA	NA
NIP2F7	127	3.000	2.674	0.693	0.626	-0.107	*
NlP2F3	127	7.000	2.790	0.181	0.642	0.718	*
NIP3G2	119	10.000	4.313	0.521	0.768	0.322	*
NIP4B1	123	2.000	1.185	0.057	0.156	0.636	*
NIP4E10	124	8.000	4.630	0.508	0.784	0.352	*
NIP4C11	120	9.000	5.791	0.650	0.827	0.214	*
NIP1A6	133	2.000	1.975	0.361	0.494	0.269	*
NIP2C5	132	5.000	3.329	0.644	0.700	0.080	*
NIP3B2	132	5.000	2.549	0.447	0.608	0.265	*
NIP3E8	127	5.000	3.737	0.504	0.732	0.312	*
Mean	125.870	5.043	2.743	0.330	0.530	0.438	*
SE	1.430	0.497	0.275	0.044	0.054	0.056	NA

Table 2. Pairwise differentiation (F_{ST}) along the river, divided the locations into distance bins that reflected samples separated from each other by i) 0–500, ii) 501–1000, iii) 1501–2000, iv) 2001–2500, v) 2501–3000, vi) 3001–3500, and vii) 3501–4000 m. Notably, the zone 1001–1500 m was excluded as only one fecal sample was collected in that section.

		0-500m	501-1000m	1501-2000m	2001-2500m	2501-3000m	3001-3500m	3501-4000m
		Female						
0-500m			0.0085	**0.02932**	0.01433	**0.03762**	0.02474	0.02557
501-1000m		0.01416		0.00895	0.01453	0.02103	0.01285	0.00865
1501-2000m	Male	-0.024571005	-0.04066		0.01333	**0.03910**	**0.02815**	0.02389
2001-2500m		0.009552452	0.02545	0.00496		**0.03190**	0.02033	0.01077
2501-3000m		0.023825369	-0.00277	-0.0228	**0.02912**		0.03837	-0.01313
3001-3500m		0.043692382	0.01264	0.03425	0.02159	0.02017		0.00115
3501-4000m		**0.059158066**	**0.05027**	**0.08786**	0.04359	0.02889	0.03311	

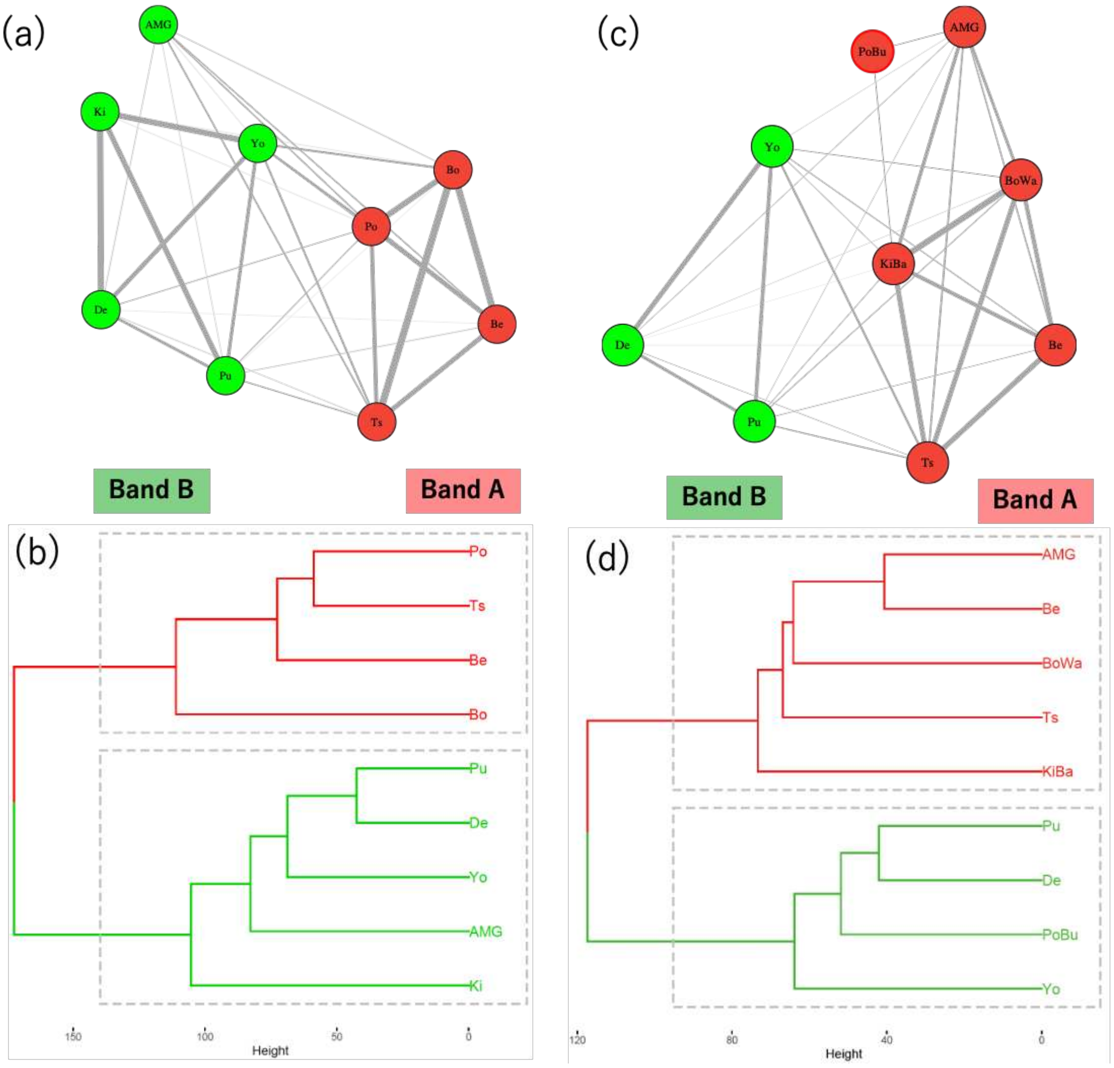

Figure 1. Network graph of the identified OMUs and AMG with cluster analyses, illustrated based on the association index from February 1999 to August 2000, when the first replacement of males in OMUs was observed (a, b), and thereafter, to June 2002, including two further replacements of males in OMUs (c, d). The two statistically estimated communities (bands) are colored.

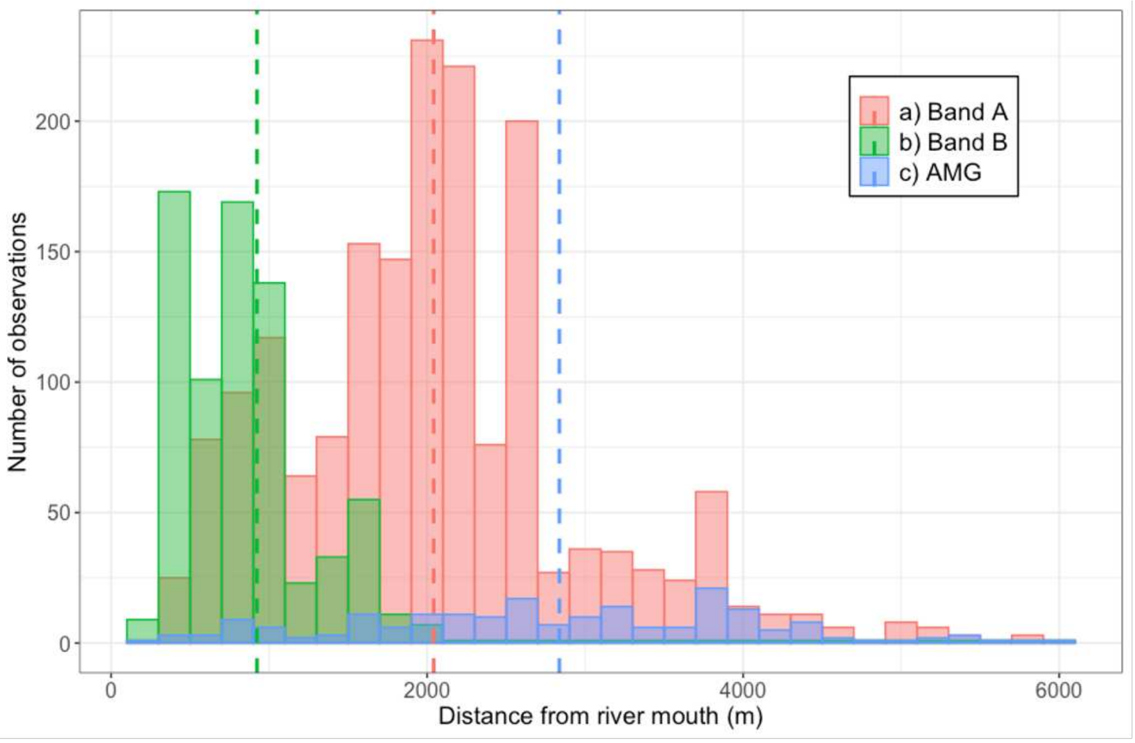

Figure 2. Distribution of sleeping sites of each band and all-male group (AMG) along the Menanggul River. The dotted lines in the graph represent the mean location of the sleeping location based on the distance from the river mouth of each band and AMG. Different bands and the AMG are colored.

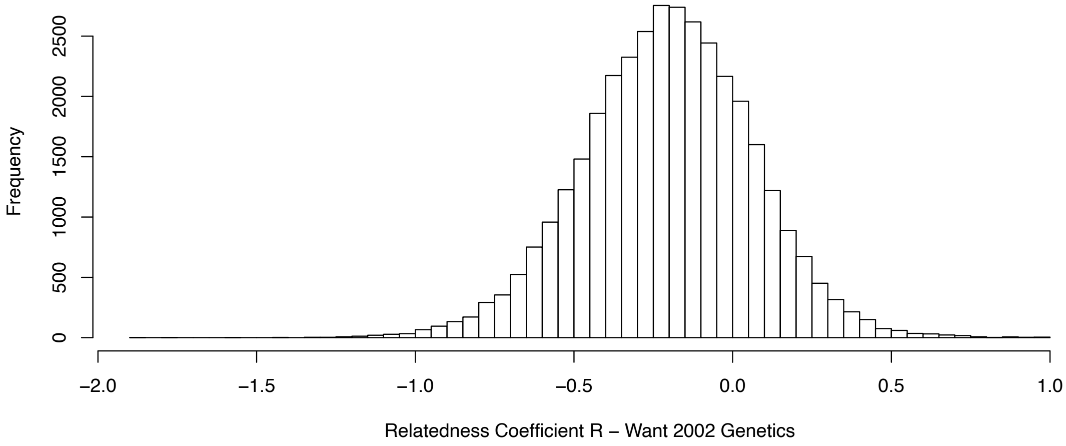

Figure 3. Distribution of relatedness pairwise comparisons measured among the 267 samples.

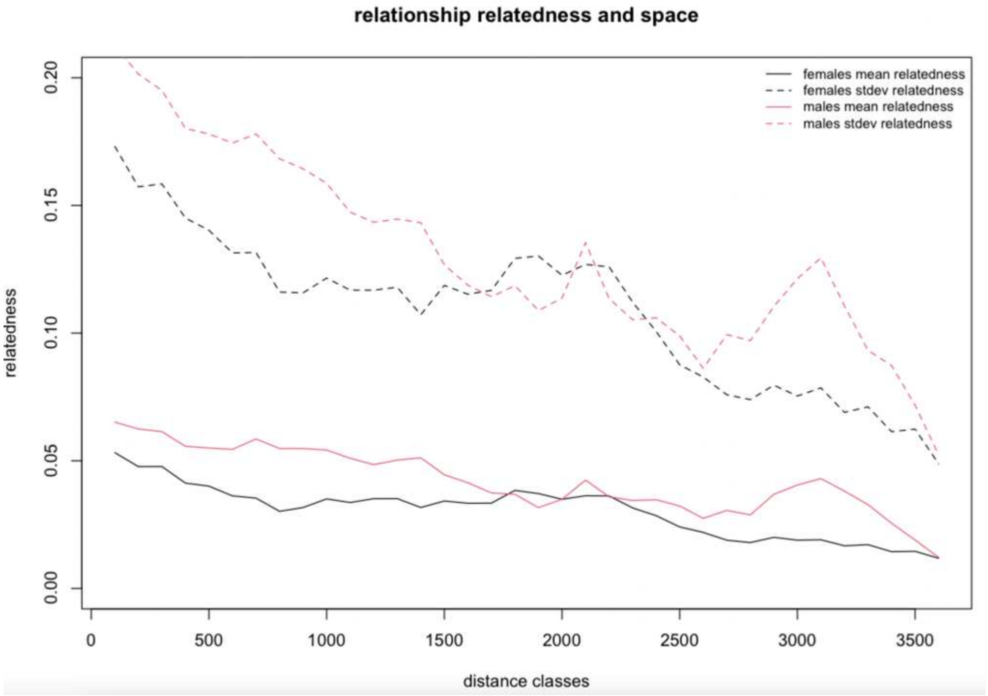

Figure 4. Relationship between geographic distance and genetic distance among the measured 267 samples.

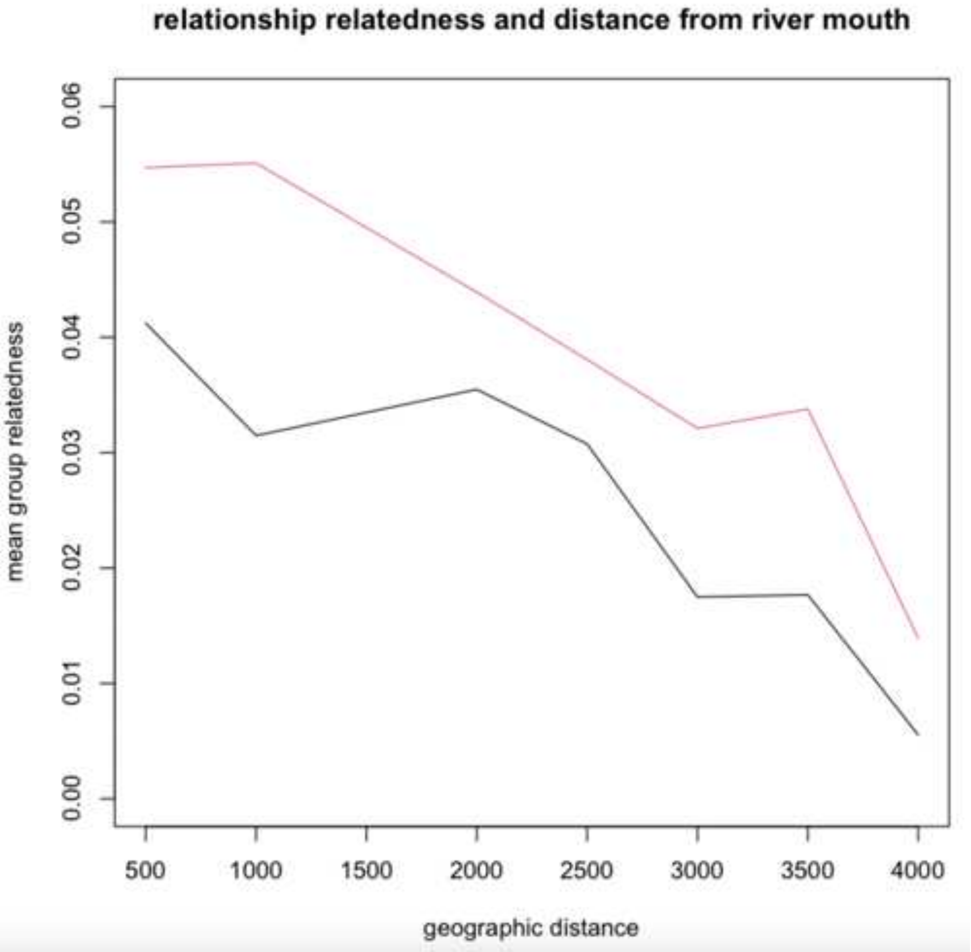

Figure 5. Relationship between the mean relatedness between zones and the corresponding geographic distance; red and black lines represent male and female mean relatedness, respectively.

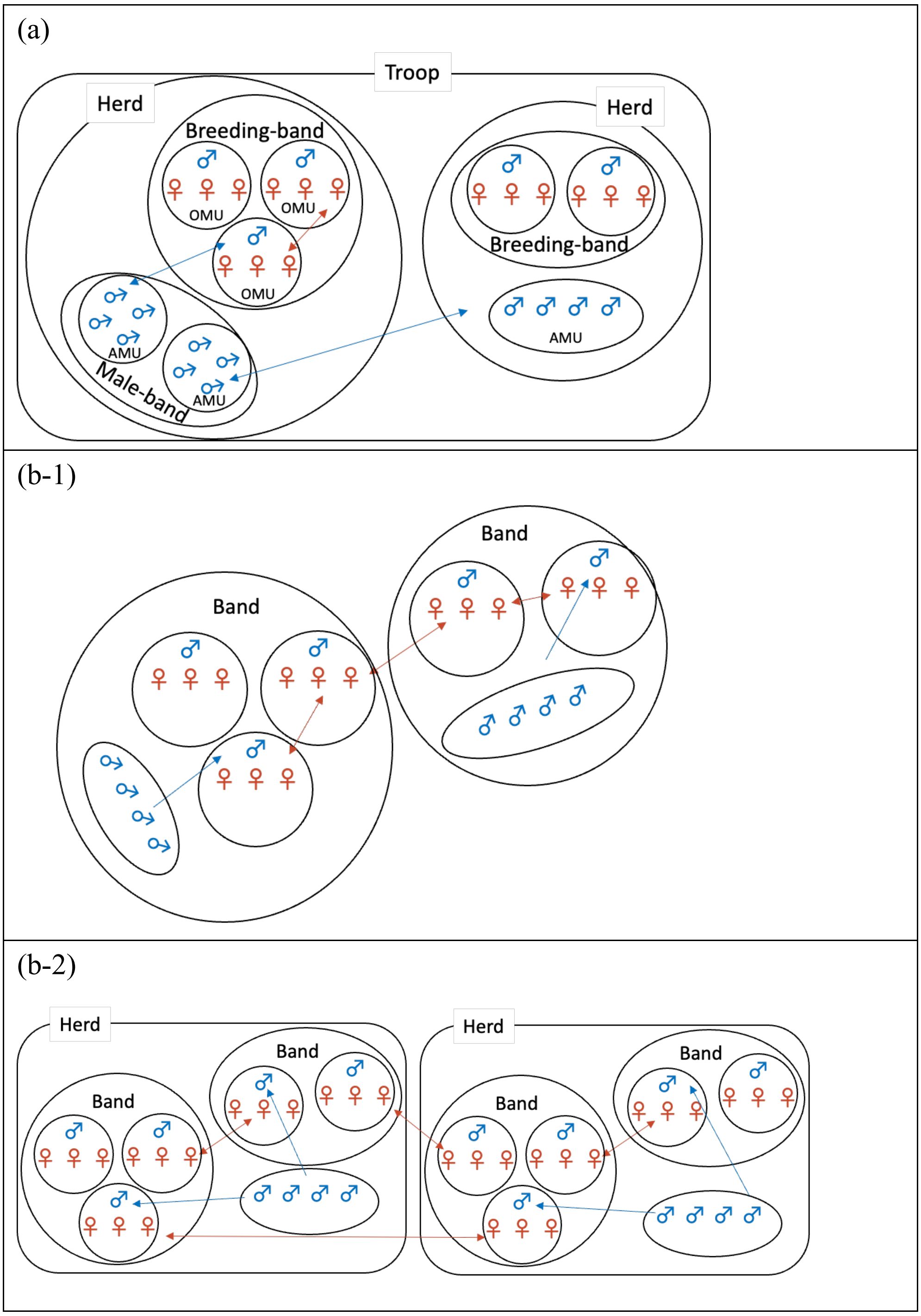

Figure 6. Schematic illustration of MLS in snub-nosed monkey (a) based on Qi *et al.* (2014) and proboscis monkeys (b); a) the “herd” level is based on the spatial proximity between a band and one or more AMUs and the “troop” level is based on the spatial proximity between two herds; b-1) MLS inferred from genetic analysis; b-2) MLS inferred from direct observation and genetic analysis. Proboscis monkeys form an MLS with a patrilineal basis within bands or herds.

Supplementary Table 1. Microsatellite marker multiplexes optimized for this study.

Multiplex Combination	**Annealing Temperature (˚C)**	**Markers used**	**Range**	**Dye**
A	60	NlE10	165-177	FAM
		NlP1C5	197-207	HEX
		NlP2B8	229-243	HEX
		NlP3B6	238-258	FAM
		NlP1C8	220-226	FAM
		NlP2C12	187-195	FAM
		DEADBOX	182-212	TAM
B	60	NlP3C11	194-201	FAM
		NlP2D6,	151-165	FAM
		NlP3B4	184-190	HEX
		NlD10	183-185	TAM
		NlP3H5	161-169	HEX
		NlP4B2	201/203	HEX
		NlP2B9	210-220	FAM
C	62	NlP2F3	175-187	FAM
		NlP3G2	232-254	FAM
		NlP4C11	245-263	TAM
		NlP4B1	152-156	HEX
		NlP4E10	207-223	HEX
		NlP2F7	138-152	FAM
D	62	NlP1A6	153-159	FAM
		NlP2C5	179-187	FAM
		NlP3B2	159-171	HEX
		NlP3E8	209-233	HEX

Supplementary Table 2. Group size and number of cases where the sleeping sites of each group were discovered.

	Group size	Number of observations
Senior OMUs		
Ts	15	326
Be	11	339
Po	18	366
Bo	15	122
Yo	8	236
De	32	93
Pu	34	197
Ki	11	24
Junior OMUs		
BoWa	15	180
PoBu	18	12
KiBa	18	223
		415
AMG	30	130