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# Genetic Evidence for Spatio-Temporal Changes in the Dispersal Patterns of Two Sympatric African Colobine Monkeys

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*ABSTRACT* Western black-and-white colobus and Temmink's red colobus are two forest-dependent African primates with similar ecological requirements, often found in sympatry. Their most striking difference lies in their social system: black-and-white colobus live in small groups with mainly male-mediated dispersal but where females can also disperse, whereas red colobus live in larger groups with males described as philopatric. To investigate whether genetic evidence supports the reported patterns of dispersal based on observational data, we examined eight black-and-white and six red colobus social groups from Cantanhez National Park, Guinea-Bissau. Microsatellite markers revealed a lack of sex-biased dispersal for black-and-white colobus. Gene flow, mainly mediated by females, better explained the genetic patterns found in red colobus, with some evidence for less extensive male dispersal. In contrast to the microsatellite data, low mitochondrial diversity for the black-and-white colobus suggests that historical and/or long-range male-mediated gene flow might have been favored. In red colobus, the co-existence of three divergent mitochondrial haplogroups suggests that the Cantanhez population contains a secondary contact zone between divergent lineages that evolved in allopatry. Female-biased dispersal in this species may be a major factor contributing to the colonization by such differentiated mitochondrial lineages in the region. Overall, we find evidence for a spatio-temporal change in the dispersal patterns of the colobus monkeys of Cantanhez, with mitochondrial DNA indicating dispersal by mainly a single sex and microsatellite data suggesting that recently both sexes appear to be dispersing within the population. Am J Phys Anthropol 000:000–000, 2013. © 2013 Wiley Periodicals, Inc.

Sex-biased dispersal has evolved in many group-living animals and is an almost ubiquitous feature of primate life-histories. Understanding a species' dispersal system is necessary for the study of its socio-ecology, population dynamics and genetic structure (reviewed in Lawson Handley and Perrin, 2007). An individual may be induced to disperse by various proximate causes such as pressure from within the group or attraction to extra-group individuals (Jones, 2003). Evolutionary theories attempt to explain the ultimate causes of dispersal and view it as a mechanism for the avoidance of negative consequences of group-living (e.g. Hamilton, 1967; Dobson 1982; Pusey, 1987; Pusey and Parker, 1987; Clutton-Brock, 1989). Dispersal can, for instance, reduce inbreeding, competition between kin for local resources and competition between mates (Lawson Handley and Perrin, 2007). Dispersal, however incurs costs to the individual. For example, lack of familiarity with a territory renders an individual vulnerable and may reduce its ability to find resources. There is also a risk of aggression from groups that the dispersing individual may encounter (Jack and Isbell, 2009). Interaction between forces that promote and oppose dispersal may explain the imbalance in dispersal rates and distances between the sexes.

Aspects of a species' biology such as sex-biased dispersal are expected to leave traces in an organism's genome (Avise, 1994; Sunnucks, 2000; Di Fiore, 2003) and can be seen through variance in allele frequencies among social groups (Altmann et al., 1996; Dobson et al., 1998;

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Gompper et al., 1998, Hammond et al., 2006). The clonal maternal inheritance of mtDNA means that for female philopatry, haplotype diversity is expected to be low within groups, due the lack of arrival of new haplotypes, genetic drift and lineage sorting (Avise, 1994; Di Fiore, 2003). For species in which females disperse, mtDNA diversity is expected to be high, due to the introduction of novel haplotypes by immigrating females. However, not only is group diversity expected to differ among dispersal modes, but also substructure patterns within populations. While both mitochondrial haplotype and microsatellite allele frequencies in female-dispersing species are expected to be homogeneous (e.g., Melnick and Hoelzer, 1992; Morin et al., 1994; Di Fiore, 2003), female-philopatry is expected to lead to high mitochondrial differentiation between groups in contrast to autosomal markers, as male dispersal will homogenize this genome throughout the population (Avise, 1994; Di Fiore, 2003). This pattern was recently found for the squirrel monkey (*Saimiri oerstedii citrinellus*) (see Blair and Melnick, 2012) where previous observational data suggested female dispersal but genetic data indicated that males might be the main dispersers, at least over longer distances. Dispersal mechanisms are not always easy to predict based on systematics alone (e.g., Faulkes et al., 1997) and can vary among closely related species as in, for example, squirrel monkeys (Boinski et al., 2005) and can even vary among populations of the same species as in the white-bellied spider monkeys (Di Fiore, 2009). The African colobines also illustrate this variation very well; besides the fact that black-and-white colobus and red colobus are known to exhibit different modes of dispersal, variation in dispersal patterns have also been reported among species of the two genera (Harris et al., 2009; Struhsaker, 2010).

Western black-and-white colobus (*Colobus polykomos*) and Temmink's red colobus (*Procolobus badius temminckii*) belong to the Old-World sub-family Colobinae. African colobus monkeys share many aspects of their ecology such as predominantly arboreal lifestyles within the forests (Oates, 1994) and dietary similarities—with most species favoring young foliage including some seeds, mature leaves, flower, and fruits (Oates et al., 1994). Nevertheless, group sizes are consistently different, with *P. b. temminckii* living in large groups, averaging 25–40, in comparison to *C. polykomos* groups with 16 or fewer individuals (Oates, 1994). Group composition between the two species also differs: red colobus groups are usually multi-male, multi-female, with a minimum of three adult males and at least twice as many adult females; black-and-white colobus groups on the other hand, consist of multiple females with often only one adult male (Oates, 1994). A major difference between the two species' social systems is their pattern of dispersal. In black-and-white colobus, although dispersal is reported to be more biased towards males, both sexes have already been described to disperse (e.g., *C. polykomos*: Korstjens et al,. 2002; *C. santanas*: Fleury and Gautier-Hion, 1999; *C. guereza*: Harris et al., 2009; *C. vellerosus*: Teichroeb et al., 2009), while in red colobus, females are the main dispersers (Marsh, 1979; Starin, 1991, 1994; Struhsaker, 2010). Female dispersal in black-and-white colobus has been explained as either a consequence of intra-group competition for resources or inbreeding avoidance (Korstjens et al., 2002, 2005; Harris 2005). As they feed on patchily distributed species within their home range, territory expansion is energetically costly. Consequently, young females may be forced to disperse in order to avoid increasing group size (Korstjens et al., 2005). These two related species (Ting, 2008) share similar ecological requirements, exhibit contrasting social systems and often live in sympatry, and are therefore good models for understanding the determinants of dispersal behavior and its impact on the genome.

Western black-and-white colobus occurs from southern Senegal to the Ivory Coast (Gippoliti and Dell'Omo, 2003), while *P. b. temminckii* occurs in Senegal, Gambia, northern Guinea and Guinea-Bissau (Oates et al., 1994). The socio-ecology of these two primates has been studied previously (*C. polykomos*: Galat and Galat-Luong, 1985; Dasilva, 1989 and 1992; Korstjens, 2001; Korstjens et al., 2005 and *P. b. temminckii*: Marler, 1970; Struhsaker, 1975; Struhsaker and Leland, 1979; Starin, 1991 and 1994), although not in Guinea-Bissau. Little is known about African colobine population genetic diversity and structure. The studies conducted so far have focused only on few colobus populations from eastern Africa (Harris et al., 2009; McDonald and Hamilton, 2010; Mbora and McPeek, 2010). Harris et al. (2009) used both observational and genetic data to describe the complex dispersal system of a *Colobus guereza* population in Uganda, where the genetic structure of social groups is shaped by male-mediated gene flow together with less common episodes of female dispersal. McDonald and Hamilton (2010) examined the genetic diversity and phylogenetic relationships among Kenyan and Tanzanian *Colobus angolensis palliatus* populations. Only one study on red colobus (*Piliocolobus badius rufomitratus*) has been reported from the Tana River in Kenya, which evaluated mitochondrial diversity within this population (Mbora and McPeek, 2010).

Here we intensively sampled several social groups for both colobus species in Cantanhez National Park, Guinea-Bissau (Fig. 1), to evaluate the effect of dispersal patterns on genetic diversity, within-population structure and historical demographic processes. Given that female dispersal is expected for both species, we predicted the two species to exhibit similar patterns of mitochondrial diversity and structure: high genetic diversity and homogenized haplotypes throughout the population. For nuclear DNA, we did not expect to find differences between sexes for *C. polykomos* as a consequence of dispersal by both males and females. However, if *P. b. temminckii* males are philopatric we would predict them to show higher genetic differentiation between social groups, higher probability of assignment to source population and higher levels of within group relatedness (Di Fiore, 2003; Hammond et al., 2006, Lawson Handley and Perrin, 2007).

## MATERIAL AND METHODS

### Study site and sampling

Surveys were conducted throughout Cantanhez National Park, in south-western Guinea-Bissau (NE limit: 11°22'58"N, 14°46'12"E; SW limit: 11°2'18"s. 15°15'58"W (WGS 84); Fig. 1), which comprises peninsular and fragmented coastal forest. Fecal samples from one social group per fragment (ranging from 47.5 to 2500 ha; Simão, 1997) were collected per species. Approximately 10 samples were collected per *C. polykomos* social group and 30 for each group of *P. b. temminckii*, in order to ensure that more than half of the group was sampled in most cases. The size and age-sex composition of each group could not be

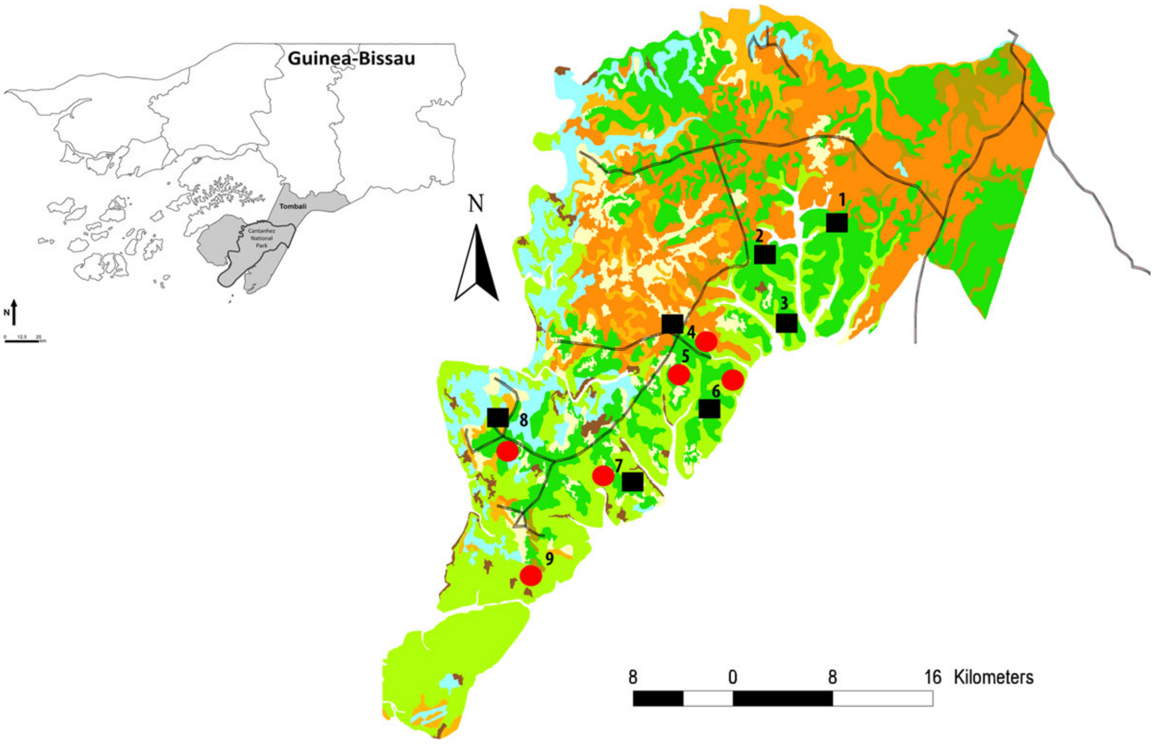

**Fig. 1.** Map of the land cover for Cantanhez National Park (provided by INEP): dark green, forest; light green, mangrove; yellow, savanna; blue, rice fields; beige, crops; brown, tannes. Black squares: black-and-white colobus sampled groups; red circles: red colobus sampled groups. 1, Cancira; 2, Amidara; 3, Deep Amidara; 4, Focal and Neighbor; 5 - Madina; 6, Cambeque; 7, Cangode; 8, Muna; 9, Cungha.

determined for non-habituated groups and we did not directly observe animals defecating but collected only fresh fecal material. To minimize multiple sampling of individuals, we only collected samples that were 2m or more apart. Samples were stored using the 'two-step' approach (Roeder et al., 2004). Samples from eight black-and-white colobus and six red colobus social groups were analyzed (Fig. 1).

## DNA extraction and amplification

DNA from 380 fecal samples was extracted using the QIAampDNA Stool Kit (Qiagen, Valencia, CA) following the manufacturer's instructions and stored at −20°C. All samples were genotyped for 15 human-derived microsatellite *loci* (Table 1), first successfully used in *Colobus guereza* by Harris et al. (2009), multiplexed in three panels of five *loci*. Molecular sex identification of each sample was carried out following the protocol developed by Villesen and Fredsted (2006) and multiplexed together with the microsatellites. PCRs contained 7.5μL Multiplex PCR Master Mix (Qiagen), 0.1μM of each primer, 0.004mg/ μL of BSA (Bovine Serum Albumin, NEB) and 4.35μL of ultrapure water, and 1.5μL template DNA in a final volume of 15μL. Reactions, including negative controls, were performed in a VeritiTM 9902 Thermal Cycler (Applied Biosystems) as follows: 30 min at 95°C; 40 cycles of 0.5 minutes at 94°C, 1.5 minutes at 55-57°C, 1.5 minutes at 72°C followed by 30 minutes at 72°C. PCR products were analyzed by Macrogen (Korea) Inc. An internal size standard (ROX labeled HD400) was added and alleles were scored using GeneMapper® v3.2 (Applied Biosystems). To account for allelic dropout, we used the simulation software GEMINI v.1.4.1 (Valière et al., 2002) that uses the allele frequencies, allelic dropout and false allele rate observed for a subset of samples, and estimates the number of PCR repeats and number of times an allele would need to be scored in order to produce genotypes with 95% confidence. As a result, each sample was positively amplified for a minimum of four independent reactions. The *locus* D2s442 was excluded from the dataset as genotypes were missing for 25% of *C. polykomos* and 20% of *P. b. temminckii* samples respectively. D12s372 was monomorphic for *P. b. temminckii* and was also excluded for this species. $PI_{sib}$ values (the probability of identity (PI) that accounts for the presence of related individuals in the sample; Taberlet and Luikart, 1999; Waits et al., 2001) were calculated using GenAlEx 6.41 (Peakall and Smouse, 2006) yielding values of 1.9 x $10^{-3}$ for *C. polykomos* and 1.2 x $10^{-3}$ for *P. b. temminckii*. Low quality DNA samples, i.e. Quality Index (QI, Miquel et al., 2006) below 0.5 or more than three missing *loci*, and samples that could be replicates from the same individual were excluded. A total of 52 *C. polykomos* individuals from eight social groups were genotyped for 11-14 *loci* with 97.2% complete genotypes and a mean QI of 0.84. In addition, 72 *P. b. temminckii* individuals from six social groups were genotyped for 10-13 *loci* with 96.5% complete genotypes and a mean QI of 0.77. We used Micro-Checker (van Oosterhout et al., 2004) to test for null alleles and FSTAT (Goudet, 2001) to conduct exact tests for Hardy-Weinberg and linkage disequilibrium. Highly related individuals, resulting from sampling social groups, had a strong effect in inducing apparent population substructure (not shown) and were subsequently removed from the dataset. After this correction, none of the *loci* showed

*TABLE 1. Diversity indices for the microsatellite loci*

| Locus | # Alleles | Allele size range (bp) | Ho[a] | He[b] | Fis[c] | Allelic R[d] |
|---|---|---|---|---|---|---|
| *C. polykomos* | | | | | | |
| D1s548 | 5 | 200–216 | 0.669 | 0.622 | –0.197 | 2.495 |
| D1s1665 | 4 | 164–176 | 0.589 | 0.537 | –0.182 | 2.265 |
| D4s2408 | 5 | 259–283 | 0.650 | 0.560 | –0.261 | 2.103 |
| D13s321 | 6 | 158–182 | 0.730 | 0.651 | –0.224 | 2.586 |
| D6s474 | 4 | 122–134 | 0.638 | 0.564– | –0.212 | 2.465 |
| D10s611 | 5 | 177–193 | 0.527 | 0.487 | –0.176 | 2.213 |
| D2s1326 | 5 | 215–239 | 0.580 | 0.590 | –0.062 | 2.381 |
| D11s2002 | 4 | 174–186 | 0.641 | 0.494 | –0.381 | 2.215 |
| D12s372 | 2 | 226–230 | 0.127 | 0.114 | –0.209 | 1.185 |
| D6s503 | 4 | 293–329 | 0.135 | 0.162 | 0.058 | 1.339 |
| D6s1056 | 3 | 265–293 | 0.414 | 0.380 | –0.180 | 1.984 |
| D10s676 | 5 | 247–267 | 0.356 | 0.630 | 0.340 | 2.376 |
| D10s1432 | 3 | 210–222 | 0.568 | 0.543 | –0.145 | 2.103 |
| Fesps | 4 | 138–158 | 0.057 | 0.056 | –0.098 | 1.122 |
| Mean | 4.2 | – | 0.475 | 0.415 | –0.138 | 2.059 |
| *P. b. temminckii* | | | | | | |
| D1s548 | 7 | 192–216 | –0.927 | 0.783 | –0.327 | 1.795 |
| D1s1665 | 5 | 187–203 | 0.382 | 0.555 | 0.225 | 1.612 |
| D4s2408 | 6 | 275–311 | 0.398 | 0.464 | 0.046 | 1.581 |
| D13s321 | 2 | 165–169 | 0.289 | 0.321 | 0.008 | 1.307 |
| D6s474 | 4 | 131–183 | 0.689 | 0.576 | –0.281 | 1.571 |
| D10s611 | 6 | 181–217 | 0.642 | 0.736 | 0.034 | 1.760 |
| D2s1326 | 7 | 215–239 | 0.836 | 0.736 | –0.247 | 1.750 |
| D11s2002 | 4 | 158–182 | 0.544 | 0.503 | –0.171 | 1.484 |
| D6s503 | 7 | 294–338 | 0.586 | 0.777 | 0.107 | 1.748 |
| D6s1056 | 3 | 269–277 | 0.327 | 0.453 | 0.138 | 1.476 |
| D10s676 | 5 | 230–266 | 0.678 | 0.688 | –0.130 | 1.745 |
| D10s1432 | 7 | 211–247 | 0.671 | 0.837 | 0.055 | 1.803 |
| Fesps | 5 | 146–162 | 0.540 | 0.561 | –0.056 | 1.613 |
| Mean | 5.2 | – | 0.538 | 0.508 | –0.045 | 1.634 |

[a] Observed heterozygosity.
[b] Expected heterozygosity.
[c] Inbreeding coefficient.
[d] Allelic richness.

evidence for null alleles, linkage disequilibrium or deviating from Hardy-Weinberg equilibrium.

We sequenced up to 478bp of the hypervariable domain (HVI) of the mitochondrial control region for 56 *C. polykomos* and 79 *P. b. temminckii*. Primers were designed using conserved regions of sequences available on GenBank for *P. b. badius* (DQ355301; Sterner et al., 2006) and *C. guereza* (AY863427; Raaum et al., 2005). Primers amplified for both species (L15449Clbs: 5' CCRCCAATACCCAAAACTGG 3', H15973Clb: 5' AGGAGAGTAGCACTCTTGTGC 3'). PCR conditions were the same as for the microsatellite except for primer concentration (2μM of each primer) and annealing temperature (63°C). Each 13μL PCR product was purified using 4μL (1:2 ratio) of Exonuclease I (10 U/μL) (USB Corp.) and Shrimp Alkaline Phosphatase 1 U/μL (USB Corp.) by incubation at 37°C for 30 min, followed by 15 min at 80°C and finally for 5 min at 12°C. Sequences were run by Macrogen Korea Inc. Forward and reverse sequences were manually checked using Sequencher v4.9 (Gene Codes Corporation) and aligned using CLUSTALW implemented in BIOEDIT 7 (Hall, 1999). Evidence that authentic mtDNA copies were sequenced instead of nuclear insertions (Numts) included observations that: multiple electrophoretic peaks were not present in the sequences; both tissue and fecal samples (in both taxa) produced the same sequences; for the black-and-white colobus, the same haplotypes were produced using two different primer sets, and; cloning and sequencing of PCR products supported the evidence that none of the sequences used in the study were nuclear copies (see supplementary material). Haplotype sequences were deposited in GenBank database (KC407944 – KC407955).

## Genetic diversity and social group structure

Genetic diversity was analyzed across all social groups through the number of alleles (N), observed ($H_o$) and expected heterozygosity ($H_e$), and unbiased allelic richness (AR), using FSTAT (Goudet, 2001). The inbreeding coefficient ($F_{is}$) was estimated using GenAlEx 6.41 (Peakall and Smouse, 2006). Analysis of molecular variance (AMOVA), using a codominant genotypic distance matrix to calculate $\Phi_{PT}$, was also implemented in GenAlEx 6.41 (Peakall and Smouse, 2006) to understand how diversity was partitioned within and among social groups. Significant tests were performed through 9999 permutations. This analysis was carried out for the social groups and for each sex separately.

Nucleotide ($\pi$) and haplotype diversity ($H_d$) were calculated for the entire species sample and for each social group. Number of polymorphic sites and mean number of pairwise differences among sequences were also estimated. Diversity measures were computed in DnaSP version 5 (Rozas et al., 2010). AMOVA, using pairwise differences, was carried using Φ-statistics as implemented in ARLEQUIN version 3.1 (Excoffier et al., 2005). For the *C. polykomos*, a two-level AMOVA (among and within social groups) was performed and for the *P. b. temminckii* we carried a three-level AMOVA out where sex was also considered. Additionally, we used BAPS (Bayesian Analysis of Population Structure) v5 (Corander and Tang, 2007; Corander et al., 2008) in order to corroborate the inference of social group structure. Here we did not incorporate geographical information and used stochastic optimization allowing K to vary between one and the total number of social groups: each analysis was repeated 10 times. The evolutionary relationships between haplotypes for both species were determined using a median-joining haplotype network calculated with NETWORK 4.5 (Bandelt et al., 1999) with $\varepsilon = 0$ and all variable sites weighted equally. Frequencies and geographic distributions of different haplotypes were used to depict geographical and potential ancestor-descendant relationships among haplotype sequences.

## Assignment tests

Mean corrected assignment indices ($m\mathrm{AI}_c$) were calculated and compared between males and females using FSTAT (Goudet, 2001) and GenAlEx 6.41 (Peakall and Smouse, 2006). The assignment index is the probability that an individual's multilocus genotype originated in the sampled population (Favre et al., 1997). Because $m\mathrm{AI}_c$ is corrected to zero, genotypes less likely to belong to the population (e.g. immigrants) are expected to show negative $m\mathrm{AI}_c$ whereas positive values are expected for natal individuals. The dispersing sex should also show higher values of variance of the corrected assignment index ($v\mathrm{AI}_c$) as a consequence of the presence of both immigrants and residents (Favre et al., 1997; Goudet et al., 2002). Because such analysis assumes that only post-dispersal individuals are present and in our case it was not possible to distinguish adults and juveniles, we used one habituated *P. b. temminckii* social group (Focal), where samples were known to belong to adult and juvenile individuals, to serve as a control. Therefore, the same analysis was run with adults and juveniles as well as just adults, and results compared. GenAlEx 6.41 was used to perform assignment tests with the *P. b. temminckii* Focal group dataset.

### Relatedness

Mean pairwise relatedness was estimated using Kingroup v2_101202 (Konovalov et al., 2004) and Coancestry v1.0 (Wang, 2011). The relatedness estimator of Queller and Goodnight (1989) was used for all possible dyads in the population and for the comparison between females and males from the same social group. Because similar results were obtained with the two programs, here we refer only to the results from Coancestry, where 1000 per-locus bootstraps were performed to achieve 95% confidence interval for each dyad. The module "Test Group Difference" was used to statistically compare mean pairwise relatedness between males and females from the same social group. Statistical significance was achieved through 1000 bootstraps for a 95% confidence interval. The test for significance was only possible for groups that have three or more individuals of each sex. Because estimators of relatedness are likely to be biased by group size (the philopatric sex might only be detected to have higher within group pairwise relatedness if the group is small: Valsecchi et al., 2002; Lukas et al., 2005) and the presence of pre-dispersal individuals increases mean pairwise relatedness for both sexes, the number of highly related dyads for each of the sexes formed by individuals from the same or different social groups were also used as an additional indicator of sex-biased dispersal. We calculated the percentage of the same sex dyads that, based on their genotypes, were statistically more likely to be parent-offspring, full-sibling, or half-sibling pairs than to be unrelated, within and among social groups given background allele frequencies in the population. In order to estimate the number of such pairs of individuals, a likelihood ratio test was implemented in Kingroup v2_101202 (Konovalov et al., 2004), where the null hypothesis of "Unrelated" was tested against the primary hypotheses of "Parent-offspring", Full-siblings and "Half-siblings". One would expect that a higher percentage of such dyads would occur within social groups for the most philopatric sex and amongst social groups for the dispersing sex.

## RESULTS

### Genetic diversity and structure

Genotypes were derived from unidentified pre- and post-dispersal individuals and diversity indices are summarized in Table 1. *P. b. temminckii* showed a slightly higher mean number of alleles and higher $H_e$ than *C. polykomos*. However, AR was higher for *C. polykomos* whereas *P. b. temminckii* showed more *loci* with a positive $F_{is}$ value. For *C. polykomos*, significant structure among social groups was found ($\Phi_{PT} = 0.165$, $P < 0.001$), although variance among individuals within the same social group explained most of the variation. This pattern was maintained when the AMOVA was conducted for females ($\Phi_{PT} = 0.184$, $P < 0.001$) and males ($\Phi_{PT} = 0.193$, $P = 0.001$) separately. Red colobus social groups also showed significant genetic variance among groups, although less than *C. polykomos* ($\Phi_{PT} = 0.057$, $P < 0.001$). While female *P. b. temminckii* exhibit similar levels of genetic structure ($\Phi_{PT} = 0.053$, $P< 0.001$), there was some evidence that *P. b. temminckii* males are the more structured sex ($\Phi_{PT} = 0.199$, $P< 0.005$).

*TABLE 2. Summary of mitochondrial DNA diversity*

| Social group | $N$[a] | # Hapl[b] | $hd$[c] | $\pi$[d] |
|---|---|---|---|---|
| *C. polykomos* | 58 | 3 | 0.16 | 0.00036 |
| Focal | 9 | 1 | 0 | 0 |
| Neighbor | 10 | 1 | 0 | 0 |
| Cambeque | 11 | 2 | 0.18 | 0.0004 |
| Cancira | 7 | 1 | 0 | 0 |
| Muna | 6 | 1 | 0 | 0 |
| Deep Amidara | 4 | 1 | 0 | 0 |
| Cangode | 3 | 1 | 0 | 0 |
| Amidara | 6 | 1 | 0 | 0 |
| Bushmeat | 2 | 1 | 0 | 0 |
| *P. b. temminckii* | 86 | 11 | 0.83 | 0.038 |
| Focal | 29 | 5 | 0.77 | 0.03 |
| Madina | 19 | 3 | 0.70 | 0.02 |
| Cambeque | 11 | 5 | 0.78 | 0.04 |
| Muna | 2 | 2 | 1.00 | 0.05 |
| Cangode | 9 | 6 | 0.89 | 0.05 |
| Cungha | 9 | 3 | 0.64 | 0.04 |
| Bushmeat | 7 | 4 | 0.81 | 0.05 |

[a] Number of sequences.
[b] Number of haplotypes.
[c] Haplotype diversity.
[d] Nucleotide diversity.

For all *C. polykomos* individuals, 478bp of the HVI domain of the control region were successfully amplified. Only three haplotypes were detected (BW1, BW2, BW3) with two segregating sites (transitions) and a mean number of nucleotide differences between two sequences of 0.17 mutational steps. Haplotype ($H_d$ = 0.17 ± 0.065) and nucleotide diversity ($\pi$ = 0.00038 ± 0.0001) were consequently remarkably low (Table 2). Haplotype BW2 was present in 51 individuals and in all but Deep Amidara social group. Haplotypes BW1 (one individual) and BW3 (four individuals) were found in one social group each (Cambeque and Deep Amidara, respectively). Four hundred and forty-eight bp of control region were successfully amplified from all 79 *P. b. temminckii*. In contrast to the *C. polykomos*, 9 haplotypes were identified, with 45 polymorphisms (42 transitions and 3 transversions). The mean number of nucleotide differences between sequences was 16.5 mutational steps, exhibiting high haplotype ($H_d$ = 0.82 ± 0.017) and nucleotide diversity ($\pi$ = 0.037 ± 0.002; Table 2). The most common haplotypes (RC1 and RC4) were present in five and seven social groups, respectively. There were four haplotypes found in only one social group (RC6-RC9).

The *C. polykomos* AMOVA for mtDNA yielded high fixation indices between social groups ($\Phi_{st} = 0.80$, $P < 0.001$), with 80% of the variation being partitioned between groups. As this population revealed low genetic diversity, this result is almost exclusively due to individuals from Amidara that exhibited an exclusive haplotype. Structure was not detected when this social group was excluded from the analysis (data not shown). BAPS analysis supported the existence of three clusters matching the three haplotypes found ($p = 0.5$, Fig. 2a). As depicted by the minimum-spanning network (Fig. 3a), BW2 was the most widespread haplotype in the population and the other two haplotypes were rare and likely to have recently originated from BW2. For *P. b. temminckii* there was weak genetic structure between social

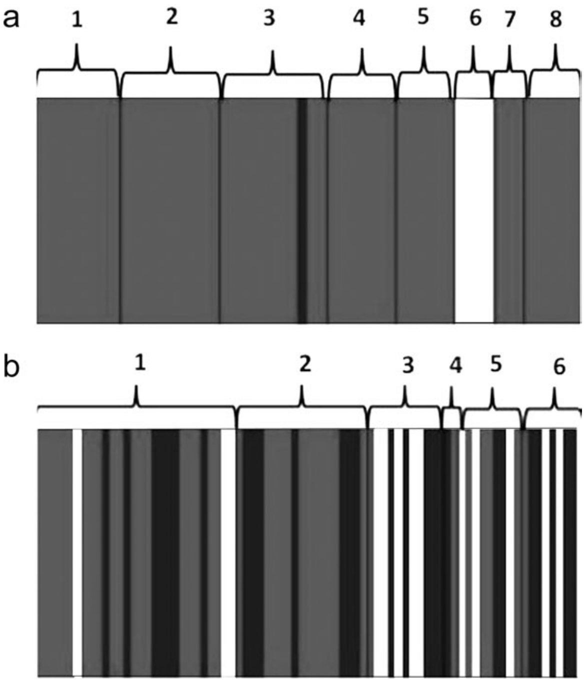

**Fig. 2.** BAPS results showing the distribution of three mitochondrial DNA haplotype clusters (indicated in the figure by white, dark gray, or black shading) across the eight social groups sampled (numbers) for black-and-white colobus (**a**): 1 Focal, 2 Neighbor, 3 Cambeque, 4 Cancira, 5 Muna, 6 Deep Amidara, 7 Cangode, 8 Amidara; and across six groups sampled for red colobus (**b**) (number of clusters in parenthesis): 1 Focal (3), 2 Madina (2), 3 Cambeque (2), 4 Muna (2), 5 Cangode (3), 6 Cungha (3).

groups (AMOVA, $\Phi_{st} = 0.13$, $P < 0.005$), with 87% of the total variation within social units. Only 16% of the variation was found within social units and none explained among males and females from the same group. The BAPS results supported the weak genetic structure found by AMOVA since the three clusters identified ($p = 0.699$, Fig. 2b) had no correspondence with social groups. Furthermore, more than two haplotype clusters were found in all social groups. All were shared between two or more social units, reinforcing the lack of mitochondrial DNA structure between *P. b. temminckii* social groups. The minimum-spanning network showing relationships between the 9 haplotypes revealed three very divergent haplogroups with a high level of haplotype sharing between social groups (Fig. 3b) in agreement with AMOVA and BAPS results.

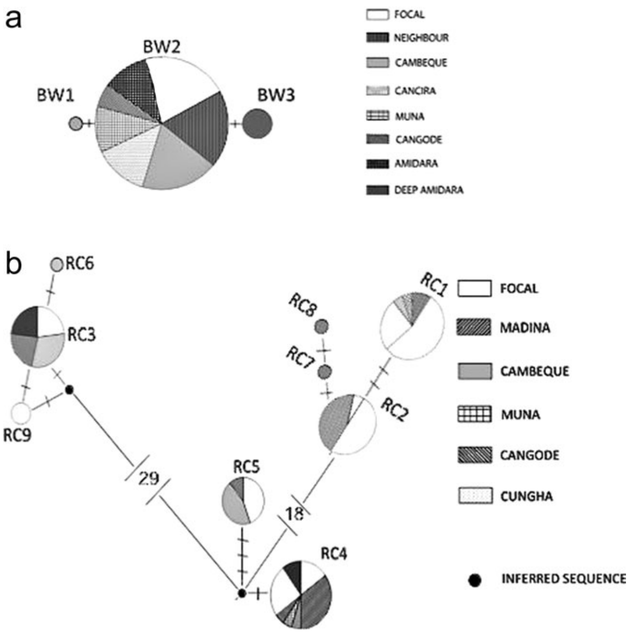

**Fig. 3.** Haplotype network for black-and-white (**a**) and red colobus (**b**) based on mtDNA d-loop sequences. Circle diameter is proportional to the frequency of the haplotypes. Different color patterns represent different social groups. Lines between haplotypes represent the number of mutational steps.

*TABLE 3. Tests of sex-biased dispersal carried in FSTAT*

| | # Individuals | $m$AIc[a] | $P$-value | $v$AIc[b] | $P$-value |
|---|---|---|---|---|---|
| *C. polykomos* | | | | | |
| Females | 30 | 0.01916 | 0.95 | 6.11120 | 0.82 |
| Males | 21 | –0.02738 | | 6.94474 | |
| *P. b. temminckii* | | | | | |
| Females | 44 | 0.22963 | 0.36 | 15.00585 | 0.52 |
| Males | 11 | –0.91851 | | 10.70524 | |

[a] Mean corrected assignment.

[b] Variance of the mean corrected assignment.

## Assignment tests

When comparing $m\mathrm{AI_c}$ values for *C. polykomos* social groups there was no strong evidence for sex-biased dispersal (Table 3). The $m\mathrm{AI_c}$ was positive for females (mean = 0.019) and negative for males (mean = −0.027). The $v\mathrm{AI_c}$ was very similar and low for both sexes. The $m\mathrm{AI_c}$ and $v\mathrm{AI_c}$ values were not significantly different between sexes. For *P. b. temminckii*, when the three social groups with both sexes (Focal, Madina, Cangode) were analyzed, $m\mathrm{AI_c}$ was positive for females (mean = 0.229) and negative for males (mean = −0.918), but the differences were not statistically significant (Table 3). However, $v\mathrm{AI_c}$ was higher for females indicating that despite a positive $m\mathrm{AI_c}$, there is greater variation in the assignment index for *P. b. temminckii* females. When the same analysis was performed for the best-studied social group (Focal), and included both adults and juveniles, a positive $m$AIc was obtained for the females (mean = 0.016) and a negative value was obtained for the males (mean = −0.050), mimicking the pattern found for the previous analysis using the three social groups. However, repeating the analysis only with Focal adult individuals, $m\mathrm{AI_c}$ values was negative for females (mean = −0.496) and positive for males (mean = 0.868), as is expected for a female-dispersal system.

## Relatedness within and among social groups

*C. polykomos* did not show a clear pattern of sex-biased dispersal when differences in within group sex relatedness were considered (Table 4). Females showed higher, but not significant, levels of mean pairwise relatedness than males for the social groups where among sex comparisons were possible (Focal: $r_{females} = 0.23$, $r_{males} = 0.07$; Cambeque: $r_{females} = 0.21$, $r_{males} = 0.07$; Amidara: $r_{females} = 0.40$, $r_{males} = 0.17$), displaying negative values for the Neighbor ($r_{females} = -0.04$) and Muna ($r_{females} = -0.23$) social groups. Additionally, a higher percentage of dyads that were more likely to be mother-daughter pairs (77.8%) or full sisters (52.4%) than to be unrelated comprised females from different social groups. Also 42% of pairs identified as more likely to be half-sisters than to be unrelated comprised females from different social groups. For males the patterns was similar: a higher percentage of pairs more likely to be close relatives than to be unrelated consisted of individuals from different social groups (father-son: 55.6%; full-brothers: 66.7%; half-brothers: 66.7%; Table 5).

Because of the limited number of *P. b. temminckii* males in this study, comparison of the mean pairwise relatedness between males and females was only possible for two social groups (Table 4). Females had higher levels of mean pairwise relatedness than males for both social groups (Focal: $r_{\text{females}} = 0.07$, $r_{\text{males}} = 0.05$; Madina: $r_{\text{females}} = 0.02$, $r_{\text{males}} = -0.14$). Again, Focal group results when only adult individuals were analyzed ($r_{\text{females}} = -0.08$, $r_{\text{males}} = 0.05$,) are more in agreement to what would be expected from a female dispersal system. Statistical tests were only possible for the Focal group and were not significant. Although 85.2% of dyads identified as likely mother–daughter pairs came from the same social group, this percentage dropped to 45.7% and 46.9% when likely full- and half-sisters were considered, respectively (Table 5). Nonetheless for males, dyads that were more likely to be father-son pairs than unrelated individuals were only found within groups, and only 28.6% of dyads identified as likely full-brothers and 16.7% of likely half-brothers comprised individuals that belonged to different social groups (Table 5).

*TABLE 4. Pairwise relatedness for males and females within each social group*

| Social Groups | R Females[a] | R Males[b] |
|---|---|---|
| *C. polykomos* | | |
| Focal | 0.22810 (4) | 0.06769 (5) |
| Neighbor | –0.04087 (4) | 0.25135 (4) |
| Cambeque | 0.20834 (6) | 0.07127 (3) |
| Muna | –0.23223 (3) | 0.09378 (4) |
| Deep Amidara | 0.10617 (4) | NA (1) |
| Cancira | 0.15390 (4) | 0.57710 (2) |
| Cangode | 0.06880 (2) | NA (1) |
| Amidara | 0.39853 (3) | 0.18690 (2) |
| *P. b. temminckii* | | |
| Focal | 0.06641 (22) | 0.05035 (8) |
| Focal adults | –0.08382 (11) | 0.05195 (4) |
| Madina | 0.01628 (13) | –0.13510 (2) |
| Cambeque | 0.00036 (10) | NA (0) |
| Muna | NA (0) | 0.39700 (2) |
| Cangode | 0.00319 (9) | NA (1) |
| Cungha | 0.24948 (5) | NA (0) |

Note: Numbers in () correspond to the number of individuals used in each class.[a] Female pairwise relatedness.
[b] Male pairwise relatedness.

# DISCUSSION

## Comparison of levels of genetic diversity and structure

The low mitochondrial diversity in the *C. polykomos* population and the high diversity found for *P. b. temminckii* are concordant with a male dispersal system in black-and-white colobus and female-mediated dispersal in red colobus. For *C. polykomos*, reduced mitochondrial gene flow as a result of female philopatry, together with the stochastic events of mutation, genetic drift and lineage sorting should result in restricted levels of genetic diversity within local populations of male dispersing species (Melnick and Hoelzer, 1992; Avise, 1995, 2000). AMOVA results show that mitochondrial haplotypes are unevenly distributed across social units resulting in 80% of the variation being explained among groups. Melnick and Hoelzer (1992) found that for the male dispersal system of *Macaca mulatta* (Rhesus monkey), mitochondrial sequence differences between populations (2.45%) were an order of magnitude larger than those within populations (0.23%), where 91% of the diversity was explained between populations. The *C. polykomos* microsatellite data showed weaker structure among social groups with most of the variation found within social units. This pattern could be concordant with what is expected for species where dispersal is mainly male mediated: female philopatry does not allow the mitochondrial genes to be distributed throughout the population, but dispersal by males homogenizes the nuclear genome (Di Fiore, 2003). However, the fact that the strong mitochondrial structure is solely due to one social group (Deep Amidara) and that the genetic diversity is extremely low makes it difficult to infer dispersal among social groups of the Cantanhez *C. polykomos* from mtDNA alone. It could be argued that forest fragmentation and not male dispersal is responsible for the fact that one haplotype is exclusive to Deep Amidara, but microsatellite data indicate that, at least currently, females from Deep Amidara are inferred to have closely related individuals in other social groups (data not shown) so emigration from this social group remains possible. In contrast, the fact that *P. b. temminckii* showed high levels of mitochondrial diversity and no genetic structure for either of the markers suggests that the females introduced novel genetic mitochondrial information in the population and were homogenizing both genomes through dispersal among social groups (Di Fiore, 2003; Lawson Handley and Perrin, 2007). In a study conducted on the closely related Asian colobine, the snub-nosed monkey (*Rhinopithecus roxellana*), one of the populations also showed comparable levels of mtDNA diversity to the red colobus population, as expected for a population where females migrate ($H_d = 0.88$ and $\pi = 0.04$; Li et al., 2007). The

*TABLE 5. Percentage of dyads of individuals of the same sex that are more likely to be closely related*

| | Parent-Offspring | | Full-siblings | | Half-siblings | |
|---|---|---|---|---|---|---|
| | Intra-group | Inter-group | Intra-group | Inter-group | Intra-group | Inter-group |
| *C. polykomos* | | | | | | |
| Females | 22.2 (4) | 77.8 (14) | 47.6 (10) | 52.4 (11) | 57.9 (11) | 42.1 (8) |
| Males | 44.4 (4) | 55.6 (5) | 33.3 (2) | 66.7 (4) | 33.3 (2) | 66.7 (4) |
| *P. b. temminckii* | | | | | | |
| Females | 85.2 (46) | 14.8 (8) | 45.7 (48) | 54.3 (57) | 46.9 (46) | 53.1 (52) |
| Males | 100 (3) | 0 (0) | 71.4 (5) | 28.6 (2) | 83.3 (5) | 16.7 (1) |

Numbers in () correspond to the number of dyads used in each class.

*TABLE 6. Summary of main results explaining the patterns of dispersal found for the two colobus populations from Cantanhez*

| | *C. polykomos* | | *P. b. temminckii* | |
|---|---|---|---|---|
| Saptio-temporal range | Dispersal sex | Genetic pattern | Dispersal sex | Genetic pattern |
| Historical/long-range dispersal (mtDNA) | Male | Extremely low genetic diversity within the population | Female | High genetic diversity within the population with three divergent haplogroups |
| Within population dispersal (mtDNA) | Not conclusive (very low diversity) | Low genetic diversity within groups<br>Social groups highly structured | Female | High genetic diversity within groups<br>Lack of structure between social groups |
| Current within population dispersal (microsatellite *loci*) | Both sexes | No sex diferences in genetic structure among social groups<br>No sex differences in within group relatedness<br>No sex differences in mAIc<br>High percentage of same sex close relatives are of individuals from different social groups for both males and females | Female but with some evidance of less extensive male dispersal | Females less strctured among social groups than males<br>Negative mAIc for adult females (Focal group[1])<br>Negative intra-group adult female relatedness (Focal group[1])<br>Most dyads of highly related females were of individuals from different social groups but most highly related males were found within groups |

[a] One social group of *P. b. temminckii* where adult and juvenile individuals are known.

same trend has also been found in other female dispersal primates, as the Proboscis monkeys (*Nasalis larvatus*; see Munshi-South and Bernard, 2011), Hamadryas baboons (*Papio hamadryas*; see Hapke et al., 2001) and bonobos, where Eriksson et al. (2004) revealed high levels of haplotype (0.78–0.92) and nucleotide diversity (0.023–0.038) where ~70% of the variation was found within sampled communities.

## Sex-biased dispersal: population- and individual-based tests

Assignment tests were not able to identify any sex bias in dispersal for the *C. polykomos* population, for which system males are thought to be the main dispersers, but where episodes of female dispersal have also been reported (Dasilva, 1989; Korstjens et al., 2002). We found no significant difference between male and female $m\mathrm{AI}_c$. In accordance, $v\mathrm{AI}_c$ was also very similar for males and females. Goudet et al. (2002) showed that tests based on $m\mathrm{AI}_c$ and $F_{st}$ are only able to detect the sex bias when this is strong. Therefore, if a sex bias in dispersal exists in this species, we can only conclude that is not strong enough to be detected by population-based methods. Further, we obtained the same indication of an absence of sex bias in the dispersal for the relatedness analysis. Females only showed higher levels of within group pairwise relatedness than males in half of the groups and we found a similar percentage of highly related females in both within and among group dyads. These results are indicative that females might disperse to some extent. Ultimately, when inspecting the distribution of dyads that are more likely to be close relatives ("parent-offspring", "full-siblings," and half-siblings"), a high percentage was found of individuals belonging to different social groups for both sexes, in agreement with the $m\mathrm{AI}_c$ and $F_{st}$ results. These results are in line with Harris et al.'s (2009) findings for a black-and-white colobus population (*C. guereza*, Uganda) where the genetic signature of the dispersal system has been studied. Their results based on pairwise relatedness within and among social groups also revealed the presence of some highly related female dyads in different social groups. However, *C. guereza* related female dyads were more likely to be found within groups and males were on average less related within groups than females. Harris et al. (2009) explained the dispersal system as being complex, where males might disperse longer distances and the less extensive female dispersal being more restricted to neighboring groups. They also explained the existence of highly related female dyads in different groups as a possible consequence of group dilution. Even if this event could explain some of the relatedness patterns found for the *C. polykomos* females in the Cantanhez population, this phenomenon alone cannot explain the extensive among group relatedness found for these females, unless it is extremely common. Moreover, female dyads more likely to be closely related were found between more pairs of groups (N pairs = 16) than male dyads (N pairs = 12) (data not shown), adding evidence for dispersal by both sexes in this population.

For the *P. b. temminckii* population, both population- and individual-based analysis indicate that females should be the main sex promoting dispersal. Although with the mean corrected assignment tests, no significant differences were found between males and females, females exhibited negative values of $m\mathrm{AI}_c$ for the Focal group when only adult individuals were considered. When this group contained both adult and juveniles, we obtained a similar pattern to the total database where the $m\mathrm{AI}_c$ was positive for the females but where the $v\mathrm{AI}_c$ was also higher for this sex. For *P. b. temminckii*, conclusions should be taken with caution as the limited number of males in the sample, can obscure the dispersal pattern (Goudet et al., 2002). Moreover, the low number of *P. b. temminckii* males did not allow an extensive comparison of within group female and male pairwise relatedness and may be responsible for an underestimation of among group "male dispersal". Nonetheless, results obtained for the well-sampled Focal group when only adult individuals are considered, suggest that females are less related than males, thus being the candidate sex to conduct dispersal, at least in this social group. The intra-specific variation of the pattern of dispersal has already been reported in primates. For example, in a well-studied group of spider monkeys (*Ateles belzebuth*; see Di Fiore et al., 2009), males were found to be more related within groups, with assignment tests also suggesting female-dispersal. This pattern was not found for a second well-studied group from a different population subject to strong anthropogenic pressure where hunting was suggested as the most likely explanation for the disruption in the groups' male relatedness pattern. If in CNP, the *P. b. temminckii* social groups of different forest fragments are under different hunting pressures, as it seems to be the case (TM, personal obs.), we cannot discard the possibility that differences in dispersal patterns among social groups exist. Nonetheless, adding to the evidence that dispersal might be mainly mediated by females in the Cantanhez *P. b. temminckii* population, is the fact that we identified no among group male-male dyads that were more likely to be related at parent-offspring level than to be unrelated, and only a few cases where males from different social groups were likely to be related at the full- or half-sib level. Clearly, more males from different social groups are needed to fully understand this dispersal system. However, our data indicate that males might not be completely philopatric as is thought for red colobus. Nevertheless, we were able to show that in *P. b. temminckii*, females should be the main sex promoting dispersal, as demonstrated by the AMOVA, assignment tests and pairwise relatedness. Adding to the evidence from the nuclear markers, the lack of mitochondrial structure among social groups and high genetic diversity for this molecular marker also supports the extensive female dispersal for red colobus.

### Current and historical or long-range dispersal

If the analysis of the mitochondrial DNA indicates historical or long range dispersal by males in *C. polykomos* and by females in *P. b. temminckii*, this signal becomes more complex when analyzing current within population dispersal. The combination of both low mitochondrial haplotype and nucleotide diversity in *C. polykomos* population suggests a scenario where the colonization of the peninsula was accomplished by one or a few mitochondrial lineages (Grant and Bowen, 1998). We suggest that the fact that the males were the primary sex to immigrate into the population may not have allowed for new mitochondrial haplotypes to be established in the population. As a consequence, historical and/or long-range dispersal mainly mediated by *C. polykomos* males is reflected not only in the present pattern of low mitochondrial diversity of the population but has also left its signature in the colonization history of the Cantanhez Peninsula. Moreover, levels of haplotype and nucleotide diversity exhibited by *P. b. temminckii* population suggest the existence of, either a large stable population with deep evolutionary history, or secondary contact between divergent lineages (Grant and Bowen, 1998). The shape of the network supports the latter since three very divergent lineages were found within the population. The coexistence of large mitochondrial differences in the same geographical area can be explained by the secondary admixture between differentiated lineages (Avise, 1987). Their immigration into Cantanhez Peninsula may reflect the species dispersal pattern during or after the colonization process.

Evidence from microsatellite data, which can be used to measure sex-biased dispersal in one generation (Lawson Handley and Perrin, 2007), shows that within the *C. polykomos* population, dispersal is mediated by both sexes, whereas for *P. b. temminckii*, females seem to be the main dispersing sex, although we have evidence of some male dispersal. The forest in Cantanhez National Park is highly fragmented and episodes of colobus being hunted for bushmeat consumption have been recorded (Minhós et al., unpublished data; Hockings and Sousa, 2011). Both species occupy patches of forest where anthropogenic pressure is high and the two main threats to their survival (habitat loss/fragmentation and poaching) co-exist. Consequently, the possibility that the colobus are changing their dispersal patterns in response to recent changes in their habitat cannot be excluded (Goossens et al., 2006). In addition, stochastic demographic events (e.g. high mortality due to hunting) might have altered within- and among-group relatedness (Di Fiore et al., 2009). This might be the case for the colobus monkeys in Cantanhez as mitochondrial DNA data indicates historical and/or long-range dispersal by one sex but nuclear data suggests that currently both sexes may disperse within the population. It is recognized that the bias in dispersal might vary with geographical scale (Lawson Handley and Perry, 2007) with males being able to disperse larger distances than females (Waser, 1985), and this black-and-white colobus might also illustrate this situation (long-range dispersal by males but within population dispersal by both sexes). The fact that most behavioral studies on black-and-white colobus species report only very few episodes of females dispersing, suggests that in those populations females disperse in a lesser extent than the males, and consequently, the female dispersal evidenced by the nuclear markers in Cantanhez may correspond to local behavioral adaptation in response to changes in the environment. Proximate causes could include inbreeding avoidance and/or kin competition for resources, as dispersing over short distances is sufficient to avoid both problems (reviewed in Lawson Handley and Perrin, 2007; Korstjens and Schippers, 2003). Ultimately, genetic data integrated in the study of dispersal patterns can provide great insights not only on the socio-genetic dynamics of a species but also on the effect that anthropogenic disturbance might have on its endangered populations.

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