



ON THE WESTERN FRINGE OF BABOON DISTRIBUTION: MITOCHONDRIAL D-LOOP DIVERSITY OF GUINEA BABOONS (*PAPIO PAPIO* DESMAREST, 1820) (PRIMATES: CERCOPITHECIDAE) IN COASTAL GUINEA-BISSAU, WESTERN AFRICA

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Abstract: Like many primate species in West Africa, habitat loss and intensive hunting are threatening the poorly studied Guinea Baboon (*Papio papio*). These factors contributed to a significant population contraction during the last 30 years. Our study presents genetic diversity estimates for the Guinea Baboon based on a 391 base pair fragment of the mitochondrial DNA D-loop hypervariable region I. We used non-invasively collected genetic samples from two locations in Guinea-Bissau: Cufada Lagoons Natural Park and Cantanhez Forest National Park. Although most sampling was opportunistic, we observed and collected samples from two dames (social units). Among the 25 sequences obtained, we found seven closely related mtDNA haplotypes and one highly different haplotype. The presence of this divergent haplotype suggests a contact area between genetically differentiated populations in Cufada Lagoons Natural Park, or dispersal of individuals. The samples gathered from both regions share two of the most common haplotypes in different frequencies, but also exhibit unique haplotypes. No significant genetic differentiation was found between social units from both regions, possibly due to common ancestral origin or frequent dispersal between sampling locations. The presence of different maternal lineages in the same social unit and a higher percentage of variation within social units suggest historical female-biased dispersal for Guinea-Bissau Baboons. We further compared mitochondrial genetic diversity of Guinea and Hamadryas Baboons. We found lower haplotype, nucleotide and theta diversity for Guinea Baboons, which points to different demographic histories of these species. This work supports the need for additional genetic studies within the full Guinea Baboon range.

Keywords: Baboon, dispersal, habitat loss, hunting, Mitochondrial DNA, primates.

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INTRODUCTION

The free-ranging populations of Guinea Baboons (also called Red Baboons, *Papio papio* Desmarest, 1820) occupy a small distribution area in western Africa (Mauritania, Senegal, Gambia, Mali, Guinea-Bissau, Republic of Guinea and Sierra Leone) when compared with other baboon species (Oates et al. 2008). This poorly studied primate exhibits great ecological plasticity (Galat-Luong et al. 2006; Patzelt et al. 2011) and inhabits a variety of habitats throughout its distribution (Galat-Luong et al. 2006).

Guinea Baboons have been listed as Near Threatened and little is known about its populations (IUCN 2013 assessed by Oates et al. 2008) (Image 1). It is acknowledged that certain local or regional populations deserve conservation measures due to their rapid decline in numbers. In several areas in western Africa only fragmented populations persist (Galat et al. 1999–2000). Excessive habitat degradation by agricultural practices (e.g. slash and burn technique), hunting and persecution by farmers, international trade of juveniles and bushmeat consumption (Wolfheim 1983; Starin 1989; Galat et al. 1999–2000; Oates et al. 2008; Minhós et al. under review) contributed to substantial range contraction during the last 30 years (Oates et al. 2008).

In Guinea-Bissau, wild populations of Guinea Baboons seem to be decreasing at a fast pace (Gippoliti & Dell’Omo 2003; Casanova & Sousa 2007; Cá 2008; Costa 2010). In the 1980s it was still possible to regularly observe troops of Guinea Baboons in most regions of the country and their range extended into the outskirts of Bissau, the capital city. After this date, the baboon conservation status in Guinea-Bissau degraded dramatically. According to the perceptions of local people (Costa 2013) and hunters (Cá 2008; Amador in press), baboons became rare and live in smaller groups or have even disappeared from some parts of the country where they were common 30 years ago. This decrease could be correlated with: (i) changes in the landscape due to extensive cashew tree (*Anacardium occidentale*) plantation, which now occupies more than 70% of the whole arable land (Barry et al. 2007); (ii) intensive hunting by military groups that have consumed baboon meat in return for salary (Casanova & Sousa 2007); (iii) bushmeat markets and restaurants that flourished in the capital and in some other smaller cities throughout the country, where baboon meat is sold and consumed along with other primate species (Casanova & Ferreira da Silva pers. obs. 2006–2010; Cá 2008; Starin 2010; Minhós et al. under review); (iv) the use of baboon skins



Image 1. Guinea Baboons *Papio papio*. Photo courtesy of A. Barata

as part of folk medicine practices (Ferreira da Silva et al. 2009; Sá et al. 2012); and (v) pet trade of very young individuals, which is a common practice throughout the country (Casanova & Sousa 2007; Hockings & Sousa 2011; Ferreira da Silva, pers. obs. 2006–2010). Baboons are believed to be relatively common in the southern part of the country (although patchily distributed) and absent from the northwest (Gippoliti & Dell’Omo 2003; Casanova & Sousa 2007; Oates et al. 2008). However, little is known about the conservation status of these persisting populations and the impact of anthropogenic related habitat modifications and hunting for pet and bushmeat trade.

One possible consequence of the rapid population decline is a reduction of genetic diversity and the isolation of sub-populations with subsequent low levels of gene flow among them, which may decrease the ability of the species to respond to future environmental changes (Frankham et al. 2002). The threats affecting baboons in Guinea-Bissau justify an assessment of the genetic diversity and degree of gene flow between persisting populations to understand their risk of extinction (Avice 2000) and to assist their future conservation and management plans.

This study describes the results of the first genetic survey on baboons in Guinea-Bissau. Sampling focussed on two of the less deforested areas (southwestern coast) within Guinea-Bissau, where baboons were reported to be present (Gippoliti & Dell’Omo 2003; Casanova & Sousa 2007). We aimed for a description of the genetic diversity and a preliminary assessment of the historical genetic structure of remaining populations. Moreover, we provide a comparison of our results with those obtained for Hamadryas Baboons (Hapke et al. 2001). This comparative analysis would help to understand the

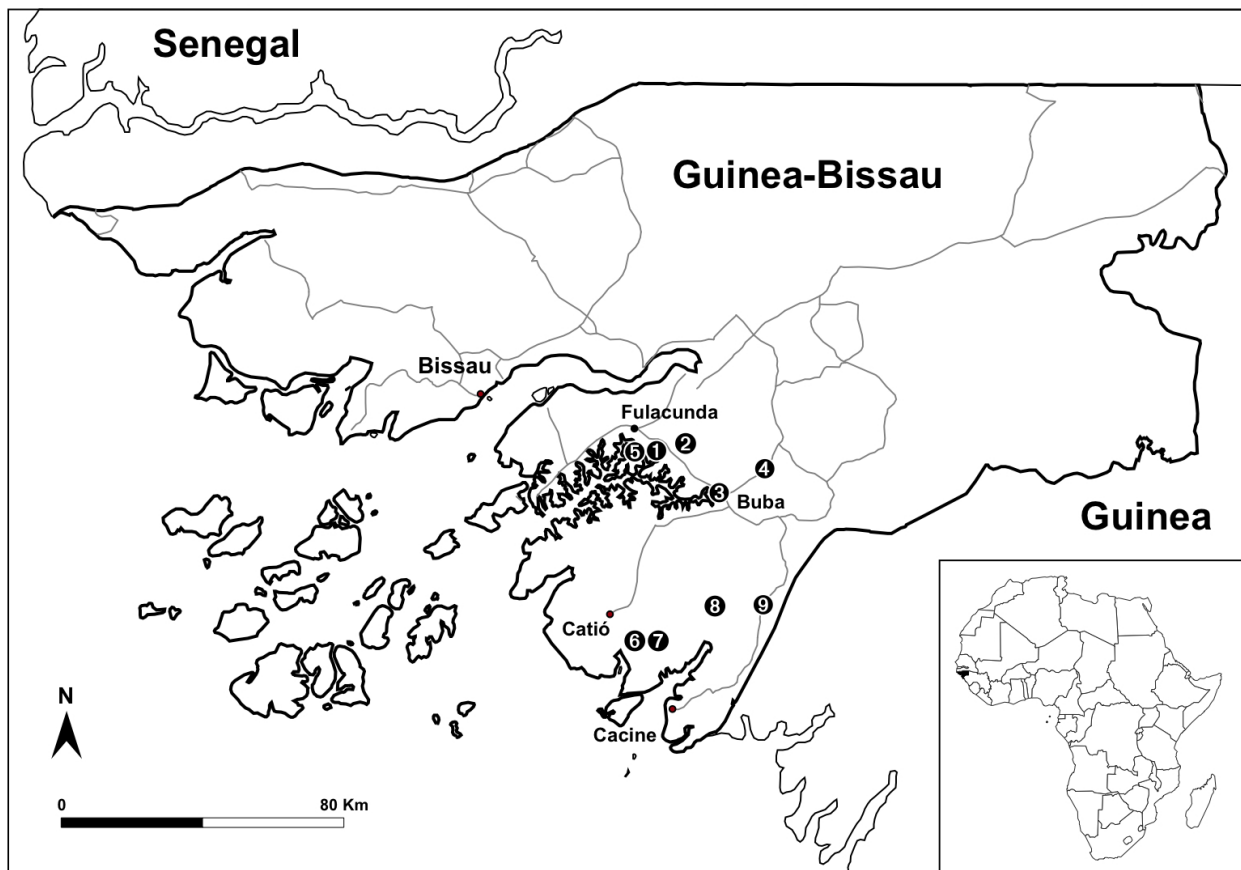


Figure 1. Location of sampling sites in Guinea-Bissau. Numbered circles refer to the sampling sites within the Cufada Lagoons Natural Park (CLNP) and Cantanhez Forest National Park (CNP) geographic regions (CLNP: from 1 to 5 and CNP: from 6 to 9, see Table 1 for details). Bottom right: location of study area within Africa.

Table 1. Number of mtDNA sequences (N) obtained from different individuals for the geographic region of Cufada Lagoons Natural Park (CLNP) and Cantanhez Forest National Park (CNP). Samples were collected in 9 sites (see correspondence with location on Figure 1). Two social units (^{SU}) were sampled. Number of amplified and sequenced samples (N), sample type and haplotypes (and respective frequency) are indicated for each site. *denotes samples collected from captive animals.

Regions (N)	Sampling sites	N	Sample type	Haplotypes (frequency)
(12)	1. Mato de Bubatchingue ^{SU}	7	Faecal	h1 (3) h2 (1); h3 (2); h4 (1)
	2. Lagoa da Cantanha	1	Faecal	h1 (1)
	3. Buba	2	Hair* and Faecal*	h1 (1); h5 (1)
	4. Boyol	1	Hair*	h1 (1)
	5. Cantanha	1	Blood	h1 (1)
(13)	6. RM	3	Faecal	h1(1); h6 (2)
	7. Caiquene ^{SU}	7	Faecal	h1 (1); h4 (3); h6 (1); h7 (2)
	8. Ponta do Rio	2	Faecal	h6 (1); h8 (1)
	9. Guilege	1	Hair*	h1 (1)

degree of genetic diversity of the Guinea-Bissau Baboon at the genus level. Our ultimate goal was to provide

baseline data for future conservation genetic studies in Guinea-Bissau Baboons.

METHODS

Sample collection and preservation

A non-invasive genetic sampling strategy was implemented as the capture of animals for collection of biological samples was considered unethical, logistically impracticable and a peril to the animal's health (Piggott & Taylor 2003). Samples were collected in the southwestern region of the Republic of Guinea-Bissau during 25 days along in the dry season (January and February 2006). Sampling was concentrated in two geographically distinct locations, 60km apart: (1) administrative region of Quinara (11°34'–11°51'N & 14°49'–15°16'W), most frequently in Cufada Lagoons Natural Park (Parque Natural das Lagoas da Cufada) (CLNP) and in (2) administrative region of Tombali (11°20'–11°05'N & 15°06'–14°4'W), predominantly in Cantanhez Forest National Park (Parque Nacional das Florestas de Cantanhez) (CNP) (Fig. 1 and Table 1 for sampling details). As no previous information was available on the location of social units in the sampling areas, we located individuals by detecting vocalizations, footprints and/or scats and used information from local people, guards and park guides. The GPS position for each sample was registered.

In 25 days of fieldwork, a total of 38 faecal samples (17 and 21 samples from CLNP and CNP, respectively) were collected. Most of the samples were collected opportunistically and were spatially scattered: we collected from one to three faecal samples in nine different sampling locations (distance within regions between 16–20 km). However, we observed and sampled two social units: Mato de Bubatchingue (MB) and Caiquene (Table 1). Faecal samples were preserved by desiccation using silica beads (type II from Sigma; Wasser et al. 1997). After one month in the field samples were transferred to 99% EtOH and preserved at -20°C prior to DNA extraction.

We collected two hair samples from captive individuals during grooming sessions and a blood sample from the carcass of a hunted animal found in the forest (Table 1). Hair samples were preserved in 99% EtOH and the blood sample was collected using an FTA card® (Whatman International Ltd, Kent, England).

This project complied with the protocols approved by CIBIO, Porto University, Portugal (for sampling of biological material and DNA extraction of blood and faecal samples) and by the School of Social and Political Sciences, Technical University of Lisbon, Portugal (for survey questionnaires and interviews). Permits for research and sample export were obtained from the

local authorities (IBAP, Institute for the Biodiversity and Protected Areas and DGFC, *Direcção Geral das Florestas e Fauna*) and research adhered to the legal requirements of the respective countries in which research was conducted.

DNA extraction and PCR amplification

Total genomic DNA was extracted from the outside surface of each faecal sample using guanidine-silica solution (Gerloff 1995) followed by DNA purification through microcon YM-30 columns (Millipore Iberica S.A.U., Madrid, Spain). DNA from hair samples was extracted using standard salting-out extraction protocols. DNA preserved in FTA card® (Whatman International Ltd) was extracted according to the instructions given by the manufacturer. Non-invasive samples were processed in a laboratory dedicated to low-quality DNA samples at the research centre CIBIO (Research Center in Biodiversity and Genetic Resources). Negative controls were always included to monitor for cross-contamination.

A 391 base pair (bp) fragment of the hypervariable region 1 (HVRI) of the mtDNA (D-loop) region was PCR amplified using primers from Hapke et al. (2001). This genetic marker was selected because it: (i) is suitable to provide an overview of the intraspecific mitochondrial genetic diversity (Wan et al. 2004); (ii) allows for a higher amplification rate on non-invasive DNA samples, when compared with nuclear DNA markers (Waits & Paetkau 2005); and (iii) made possible a comparison of our results with those obtained for *Hamadryas Baboons* (Hapke et al. 2001), one of the few available databases of D-loop sequences for any baboon wild population.

The PCR mixture contained 0.5µM of each primer, 4 µM of each dNTP, 3% DMSO, 1X PCR reaction buffer (with 1.9 mM MgCl₂), 1U of Phusion™ High-Fidelity DNA Polymerase (Finnzymes, Vantaa, Finland) and 2µl of DNA template in a total volume of 10µl. Amplifications were performed using a touchdown PCR protocol with an initial denaturation step at 98°C for 30 sec. This was followed by 10 cycles of 10 sec at 98°C, 30 sec at 58°C and 15 sec at 72°C, with the annealing temperature decreasing by 0.5°C each cycle, plus 30 cycles of 10 sec at 98°C, 30 sec at 53°C and 15 sec at 72°C, with a final 5 min extension at 72°C. To monitor for contaminations, all PCR reactions included a negative and a positive PCR control, as well as the negative control from the DNA extraction. The amplicons were sequenced using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, California, USA) on an ABI 3130xL Genetic Analyser (16 capillary sequencer, Applied Biosystems). Samples that exhibited the same mtDNA haplotype

in each location were genotyped for three human microsatellite loci (D10S611, D7S503 and D5S1457) known to be polymorphic in baboons (Bayes et al. 2000) to test for possible repeated sampling of individuals.

We used a molecular protocol to identify the sex of individuals (described in Ferreira da Silva 2007) and screened 22 faecal samples across five PCR replicates.

Data analysis

Sequences were manually checked for accuracy and aligned in BIOEDIT 7.0.9 (Hall 1999). Genetic diversity indices were calculated for the entire sequence set and for the sequences obtained from the defined social units (MB and Caiquene) using ARLEQUIN 3.5 (Excoffier & Lischer 2010). The number of variable sites, the number of pairwise differences between pairs of sequences, haplotype diversity (H_d ; Nei 1987) and nucleotide diversity (π ; Nei 1987) and their respective variances were calculated for the entire sequence set.

Genetic relationships among haplotypes were described by a median-joining network constructed using NETWORK 4.2.0.1 (Bandelt et al. 1999; www.fluxus-engineering.com). For this analysis, indels were included and all sites were treated with equal weights. Two sequences available at GenBank were included in the alignment for reference, one from *P. hamadryas* and the other from *P. papio* (Accession numbers AF275457 and AF275383, respectively).

Analysis of genetic structure was performed only with sequences from the two social units (MB and Caiquene, $N=14$). This option prevented a possible bias in the results of genetic differentiation caused

by the admixture of several social units within each geographic region. However, since some individuals of these social groups were possibly not sampled, the number of haplotypes found in each social group can be underestimated. We performed an Analysis of Molecular Variance (AMOVA analysis, Excoffier et al. 1992) using ARLEQUIN 3.5 (Excoffier et al. 2010) to assess the extent of differentiation among populations (calculating ϕ_{ST} , ϕ_{CT} and ϕ_{SC}) following a non-parametric permutation approach (10,0172 permutations).

The final dataset in our study was compared to results obtained from Hamadryas Baboons (Hapke et al. 2001). Since genetic diversity within social groups can be influenced by the dispersal rate (Rogers 2000), we selected pairs of Hamadryas social groups or demes located at a similar distance to that between the two social groups sampled for Guinea Baboons (i.e., $N=18$ pairs of "troops", located between 50–100 km). It is assumed that the ability to disperse (i.e., the organism's movement from the natal area to the breeding area) over such a distance is the same for both species. The pairs of selected demes from Hapke et al. (2001) were as follow: Furrus-Abdur, Furrus-Dogali, Furrus-Debresina, Furros-Geleb, Furros-Molki, Durfo-Abdur, Durfo-Dogali, Durfo-Molki, Durfo-Barka River, Arborobo-Abdur, Arborobo-Geleb, Arborobo-Molki, Abdur-Dogali, Dogali-Kubkub, Debresina-Molki, Debresina-KubKub, Geleb-Kubkub and Molki-Barka River. D-loop sequences were downloaded from GenBank (Accession Numbers AF275383 to AF275475) and haplotype and nucleotide diversity per site and the theta (θ) were calculated for each selected pair using DNASP 4.10.9 (Rozas et al. 2003). Finally, these

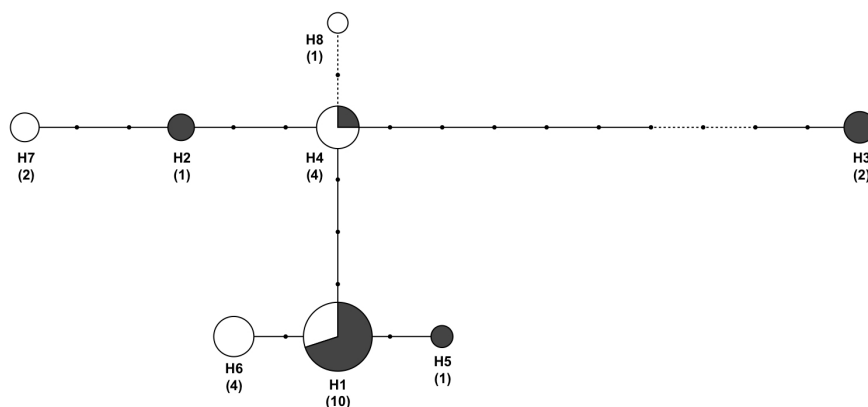


Figure 2. Median-joining network showing relationships between haplotypes (constructed using NETWORK 4.2.0.1). The Haplotypes are represented by circles with sizes proportional to respective frequency and absolute frequencies are indicated by number in parentheses. The mutation steps between the haplotypes are represented by small black dots along the lines. The branch lengths are proportional to the number of mutational steps separating haplotypes. All sites were treated with equal weights. Indels were included in the network analysis (and are represented by dashed lines). Note that if indels were not taken into account, haplotype h8 would be identical to haplotype h4 and haplotype h3 would be separated by one less mutation step from h4. The proportion of haplotypes found in both regions are indicated by different colours: black for Cufada Lagoons Natural Park (CLNP) and white for Cantanhez Forest National Park (CNP).

values were compared to the results from our study.

RESULTS

DNA extraction and amplification

Extraction of DNA was attempted from all samples collected. We successfully amplified 31 of the collected faecal samples (82%), the two hair samples and the blood sample for the 391 bp fragment of the mtDNA D-loop. Despite the high amplification rate achieved for faecal samples, good quality sequences were only obtained for 26 out of the 31 amplicons. Two samples in Mato de Bubatchingue and two samples in RM exhibited the same haplotype and the same genotype for the three microsatellite loci. We removed from the dataset one sample per location to prevent repetition of individuals. Using the sex identification protocol, only 14/22 faecal samples gave consistently the same result three times. We effectively distinguished six females and eight males among our faecal samples of Guinea Baboons.

D-loop diversity of coastal Guinea Baboons

Sequencing of 391bp of the mtDNA D-loop region distinguished eight haplotypes from the 25 sampled individuals ($H_d=0.803\pm 0.061$). Eighteen out of 391 nucleotide sites analyzed were polymorphic and estimated nucleotide diversity (π) was 0.0104 ± 0.0059 . Sixteen of the sites showed substitutions, with transitions exceeding the number of transversions (15:2). Additionally, two indels were observed: the deletion of one nucleotide in two sequences and the insertion of one nucleotide in one sequence. The mean number of pairwise differences between haplotypes (indels taken into account) was 4.06 ± 2.1 .

The median-joining network built with the eight haplotypes displays two most common haplotypes (h1 and h4), which differ by three positions and are shared by both CLNP and CNP geographical regions but in different frequencies (h1: 28% and 12%, and h4: 4% and 12% in CLNP and CNP, respectively) (Fig. 2). Five other haplotypes were found in lower frequencies (h2, h5, h6, h7 and h8) and are separated by one or two mutational steps from each other and from h1 and h4. The haplotype h8 is separated by an indel (nucleotide insertion) from the haplotype h4. An additional haplotype (h3) was observed in the CLNP sample. This haplotype h3 is separated from h4 by eight substitutions and one indel (nucleotide deletion), which corresponds to a genetic divergence of 2.3% from the central haplotype h4. Both geographic regions exhibited unique haplotypes (h2, h3

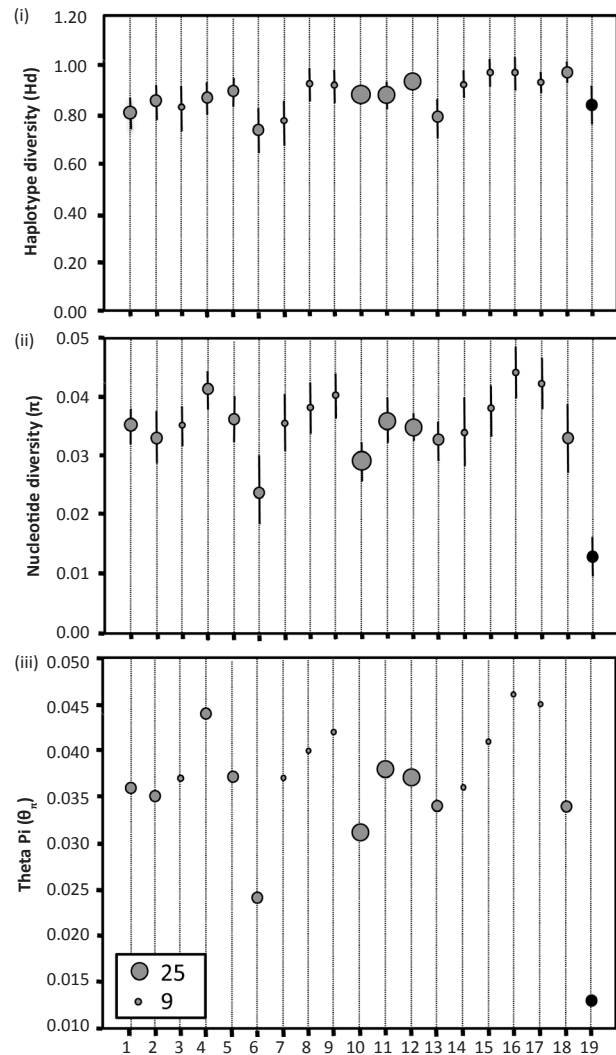


Figure 3. Comparison of genetic diversity indices between Hamadryas and Guinea Baboons. The X-axis represents pairs of demes (social units) which were separated by 50 to 100 km. (i) Haplotype diversity \pm standard deviation; (ii) Nucleotide diversity \pm standard deviation; (iii) Theta Pi (θ_{π}). Grey symbols correspond to hamadryas baboons and black symbols to Guinea baboons. The sizes of the circles are proportional to the sampling effort (the circles area representing 25 sequences and 9 sequences are showed for reference). 1 - Furrus-Abdur, 2 - Furrus-Dogali, 3 - Furrus-Debresina, 4 - Furros-Geleb, 5 - Furros-Molki, 6 - Durfo-Abdur, 7 - Durfo-Dogali, 8 - Durfo-Molki, 9 - Durfo-Barka River, 10 - Arborobo-Abdur, 11 - Arborobo-Geleb, 12 - Arborobo-Molki, 13 - Abdur-Dogali, 14 - Dogali-Kubkub, 15 - Debresina-Molki, 16 - Debresina-KubKub, 17 - Geleb-Kubkub, 18 - Molki-Barka River, 19 - MB-Caiquene

and h5 for CLNP and h6, h7 and h8 for CNP; Fig. 2).

Genetic diversity and differentiation of social units

The two social units showed the same haplotype diversity ($H_d=0.810\pm 0.130$) and both displayed the same number of haplotypes ($n=4$) (Table 1). The MB sample had slightly higher nucleotide diversity ($\pi_{MB}=0.0157\pm 0.0035$)

than the Caiquene sample ($\pi_{\text{Caiquene}} = 0.00928 \pm 0.0021$). The two social groups were tested for statistically significant genetic structure using an AMOVA analysis, which showed no phylogeographic structure of haplotypes: 7.75% of the total variance was found between social groups whereas most of the variance (92.25%) was present within social groups. Accordingly, the fixation index estimated between Guinea Baboon social groups was low ($F_{st} = 0.078$) and did not differ significantly from zero ($p = 0.2$).

Comparison of mitochondrial variation between Hamadryas and Guinea Baboons

The number of haplotypes found in this study was, on average, similar to the number found in different demes of *P. hamadryas* ($N_{h_{\text{HAM}}} = 6$ and $N_{h_{\text{GUI}}} = 8$) with similar average sampling efforts ($N_{\text{seq}_{\text{HAM}}} = 14$ and $N_{\text{seq}_{\text{GUI}}} = 14.89$). Nevertheless, all three other genetic measures were lower for Guinea Baboons social units, as documented for haplotype diversity (average $Hd_{\text{HAM}} = 0.886$ and $Hd_{\text{GUI}} = 0.846$, Fig. 3i), average nucleotide diversity found per site ($\pi_{\text{HAM}} = 0.0356$ and $\pi_{\text{GUI}} = 0.01297$, Fig. 3ii) and Theta based on π ($\theta\pi_{\text{HAM}} = 0.037 \pm 0.0053$ and $\theta\pi_{\text{GUI}} = 0.013$, Fig. 3iii), suggesting lower effective population size for Guinea Baboons.

DISCUSSION

The present work estimated for the first time the mitochondrial genetic diversity levels for wild Guinea Baboons in Guinea-Bissau and presented an assessment of the historical genetic structure of two populations.

Genetic diversity of baboons in Guinea Bissau ($Hd_{\text{GUI}} = 0.803 \pm 0.061$; $\pi_{\text{GUI}} = 0.0104 \pm 0.0059$), according to Grant & Bowen (1998), may be interpreted as a result of a large and stable population over a long period of time or as the occurrence of a secondary contact zone between previously differentiated lineages ($Hd > 0.5$ and $\pi > 0.5\%$, Grant & Bowen 1998). In particular, the presence of a very distinctive haplotype (h3) harboured by two individuals in a homogenous deme (social unit Mato de Bubatchingue) suggests that the hypothesis of a contact zone between two genetically divergent populations in CNLP (Avisé et al. 1987) best explains the observed results. Alternatively, past or contemporary dispersal from other populations can explain the presence of h3.

Although the amplification of nuclear pseudogenes can be common in studies of primates using mtDNA as a molecular marker (Mourier et al. 2001; Thalmann et al. 2004), we excluded the possibility that h3 was a numt.

Throughout our study and in particular in the samples harbouring h3 (extracted from faecal samples), we did not see ghost bands in agarose gels when testing for DNA amplification or double peaks in chromatograms (Bensasson et al. 2001). Moreover, sequences did not show any ambiguities, either when comparing both sequence strands or when comparing polymorphic sites between sequences in the final alignment (Bensasson et al. 2001). We also believe that obtaining no sequencing product for some amplicons was related to low DNA quality/quantity and not with mutations at the flanking regions, as Hapke et al. (2001) designed primers in highly conserved regions.

No evidence was found for significant geographic structure of haplotypes, and we propose that baboons present in this area of Guinea-Bissau do not represent two genetically differentiated populations. This result suggests a common origin of CNP and CLNP populations or dispersal of individuals between the two sampling locations. Guinea Baboons are able to move about 40 km a day (Galat-Luong pers. comm. January 2007), which almost equals the distance between the two sampling sites in Guinea-Bissau (about 60 km; see Fig. 1), and they occupy broad home ranges in Senegal (25 km², Fickenscher 2010).

A historical female-biased dispersal pattern for the Guinea Baboon can explain the presence of very different maternal lineages in the same social unit (see results for CLNP social unit, Mato de Bubatchingue) and higher percentage of variation within social units (Di Fiore 2003). This hypothesis would contrast with the proposal of unique maternal family groups for the Senegalese population made by Sharman (1981) based on observational data but it agrees with evidences for male philopatry based in nuclear DNA data provided by Fickenscher (2010). Fickenscher (2010) found significantly higher F_{st} values and a higher negative correlation between pairwise relatedness and geographic distances for males when compared with females, which imply a scenario of female-biased dispersal (Fickenscher 2010). Furthermore, in *Papio* male-biased dispersal species (e.g., Gray-footed Baboons, Burrell 2008) it is common to observe the inverse pattern of D-loop mtDNA variance found for the Guinea Baboon (i.e., greater percentage of variance between social units than within) and significant values of F_{st} between social units (e.g., $F_{st_{\text{gra}}} = 0.757$, Burrell 2008).

Moreover, it was found (Ferreira da Silva et al. unpublished data) that the haplotype networks of Guinea and Hamadryas Baboon social units display a similar structure: two divergent groups were present in

most Hamadryas Baboon groups, which resembles the haplotype structure found in Guinea Baboons social units by this study, although with a much lower degree of differentiation between the two most frequent haplotypes (h1 and h4). Hamadryas Baboons' display a pattern of female-biased dispersal (Hapke et al. 2001; Hammond et al. 2006; Handley et al. 2006) and the social organization of Guinea Baboons and Hamadryas Baboons share some characteristics (Galat-Loung et al. 2006; Patzelt et al. 2011). Therefore is possible that the similarity between haplotype networks of Guinea and Hamadryas Baboon social units is related with female-dispersal, although such proposition needs to be further investigated.

The current work suggests lower mitochondrial genetic diversity (both nucleotide diversity and $\theta\pi$) for Guinea-Bissau Baboons when compared with Hamadryas Baboons. Additionally, the mean number of pairwise differences estimated for Guinea Baboons (4.06, range 12-1) is rather low when compared with those observed in Hamadryas Baboons (12.40, range 23-1; Hapke et al. 2001). Moreover, comparing our results ($\pi_{\text{GUI}}=0.0104\pm 0.0059$) with Kinda, Yellow and Gray-footed Baboons mtDNA genetic diversity estimates ($\pi_{\text{KIN}}=0.036$, $\pi_{\text{YEL}}=0.086$ and $\pi_{\text{GRA}}=0.053$, Burrell 2008), the pattern of lower nucleotide diversity for Guinea-Bissau Baboons is still noticeable. Reasons that could explain such differences on genetic diversity levels include: (i) a strong stochastic event that would affect the D-loop region, as a selective sweep (Galtier et al. 2000); (ii) a past population bottleneck, which would result in a decrease in nucleotide diversity, followed by a population expansion early enough to generate the same number of haplotypes but not large differences among them (Grant & Bowen 1998); or (iii) a founder effect, which could be a consequence of a rapid colonisation of western Africa by this species (Zinner et al. 2011) or re-colonisation events by few or genetically similar individuals.

Disentangling among the hypotheses posed by this initial genetic survey will be a future challenge. With the use of more variable molecular markers (e.g., microsatellites, Wan et al. 2004) it will be possible to obtain a broader idea of genetic diversity patterns at different genome compartments and to perform studies on the demographic history of baboons in Guinea-Bissau. Additionally, if confirmed the presence of baboons in other areas within the country (e.g., Boé sector, in the southern part, see Casanova & Sousa 2007), the inclusion of samples from a wider area would allow the assessment of the degree of functional connectivity between all persisting populations. Despite the above-

mentioned limitations, our study is the first approach to estimate genetic diversity of baboons in Guinea-Bissau and one of the very few comparing mtDNA patterns of different species of *Papio*.

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Portuguese Abstract: À semelhança de outras espécies de primatas da África Ocidental, a perda de habitat e a caça intensiva ameaçam o Babuíno da Guiné (*Papio papio*), um primata raramente estudado no passado. Estes factores de ameaça contribuíram para uma redução populacional significativa nos últimos 30 anos. O nosso estudo apresenta estimativas de diversidade genética para o Babuíno da Guiné que foram baseadas num fragmento de 391 pares de bases do DNA mitocondrial, da região hipervariável I do D-loop. Foram usadas amostras não-invasivas recolhidas em duas regiões da Guiné-Bissau: o Parque Natural das Lagoas da Cufada e o Parque Nacional das Florestas de Cantanhez. Embora a amostragem tenha sido de natureza oportunística, foram observados e amostrados dois demes (grupos sociais). A análise das 25 sequências obtidas revelou sete haplótipos geneticamente próximos e um haplótipo muito diferenciado. A existência deste haplótipo muito divergente sugere uma zona de contacto entre populações geneticamente diferenciadas ou a presença de indivíduos imigrantes na Cufada. As áreas amostradas partilham dois dos haplótipos mais comuns, embora em frequências diferentes, e exibem haplótipos únicos. Entre os grupos sociais não foi encontrada diferenciação genética significativa, possivelmente devido a uma origem ancestral comum das regiões ou a dispersão frequente de babuínos. A presença de linhagens matrilineais distintas e a elevada percentagem de variação mitocondrial nos grupos sociais sugere dispersão histórica mediada por fêmeas nos Babuínos da Guiné da Guiné-Bissau. Adicionalmente, foi comparada a diversidade genética mitocondrial entre os Babuínos da Guiné e os Babuínos Hamadryas (Eritreia). Os Babuínos da Guiné apresentaram diversidade haplotípica, nucleotídica e Theta Pi mais baixa do que os Babuínos Hamadryas, o que sugere diferentes histórias demográficas para as espécies comparadas. O nosso trabalho suporta a necessidade da realização de estudos genéticos adicionais usando amostras recolhidas em toda a área de ocorrência da espécie.

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