

Hunting pressure and the population genetic patterns and sex-mediated dispersal in the Guinea Baboon in Guinea-Bissau

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Dedication

To my grandfather Luis Dias and my grandmothers Maria Adelaide Reis and Ana da Silva. I wish you could have seen the results of your affection and confidence!

To my mother Augusta and father Jorge, with love.

In memory of Cândido Magalhães.

Abstract

In Guinea-Bissau (GB) the Guinea baboon (*Papio hamadryas papio*) is threatened by hunting pressure. Along with local extinctions, these practices may be inducing long-term genetic changes and disrupting underlying social structure. In this study, the bushmeat trade in GB was evaluated for the first time and the effect of hunting practices on the genetic diversity and population structure was investigated. By following the bushmeat trade at urban markets, we found baboons to be the third most traded primate species. Male baboon carcasses were sold at a price 60% higher than any other primate due to their larger body mass. Semi-structured interviews conducted with hunters revealed a preference towards male baboons and recent difficulty in finding this primates species. Non-invasive DNA sampling in southern GB and two different genetic markers (fourteen microsatellite loci and a fragment of the mitochondrial control region) suggested substantial levels of genetic diversity and recent genetic contact between different populations. However, geographic distances had a weak effect on population structure and the genetic discontinuities found were not related with landscape features. A contact zone was identified. Here, gene flow seems to be unidirectional and admixed individuals were in higher proportion. Hunting pressure may have induced recent contact between genetically differentiated individuals, which now co-exist in the same social unit. Additionally, the sex-specific patterns of gene flow and the composition of social units were compared with a non-hunted Guinea baboon population, using a molecular sex determination protocol and thirteen microsatellite loci. GB displayed a lower ratio of males within social units, which are formed in some cases by unrelated individuals. The clear female-biased dispersal pattern displayed in Senegal was less intense in GB, where gene flow seems to be mediated through both sexes. The aforementioned contact zone resulted from male immigration. Male baboon dispersal in GB could be the result of flight behaviour or a consequence of an altered sex ratio induced by hunting practices. The GB baboons displayed signs of a disrupted population and its future conservation requires specific actions to reduce or eliminate this activity.

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Chapter 1 Introduction

Pe di tras na djubi pe di dianti

(The foot at the back looks at the foot in the front -

Guinean-Bissau popular saying suggesting the young ones imitate the behaviour of the
oldest)

1.1 - Foreword

Contrary to other primate species, baboons (*Papio hamadryas spp.*) are thought to persist in areas with some degree of habitat modification or in human-dominated landscapes. They can take advantage of living beside human settlements, by including human-derived food in their diet or by becoming crop raiders. As a consequence, baboon populations often suffer direct persecution and can be reduced or exterminated. This is the case for Guinea baboons (*Papio hamadryas papio*): their populations are disappearing in a number of locations within its small range due to a combination of anthropogenic factors such as habitat loss and hunting pressure.

Along with contraction in size, hunting pressure can cause significant long-term genetic changes to populations. Harvesting can change the pattern of gene flow between demes, contributing to a greater isolation and loss of genetic diversity even if the habitat remains unchanged. Additionally, immigration from neighbouring areas will obscure the hunting-driven demographic decline. Social species, in particular, can suffer from a disruption in social structure, which might induce changes in social group's behavioural and genetic pattern.

The population genetic changes induced by hunting pressure are poorly understood in primates, even though this is a relevant threat affecting many species. In this study, the effects of hunting pressure on the genetic diversity and population structure of a Guinea baboon population are investigated. Non-invasive DNA samples were collected in Guinea-Bissau, a small country in West Africa, where baboons are primarily hunted for bushmeat consumption. By using a comparative analysis with a Senegalese non-hunted population, I aim to describe genetic patterns and processes induced by hunting practices in populations of a generalist primate species. This research aims at a better understanding of the threat factors affecting Guinea baboons and in turn, to contribute to its conservation.

Since there is limited amount of knowledge about Guinea baboons, the general features of the genus *Papio* are reviewed here, including taxonomy, phylogeny and evolutionary history, ecology, social organization and patterns of genetic diversity and population structure. Where known, information specific to Guinea baboons is included. Additionally, the major threats affecting baboon populations, including in Guinean Bissau, are described. Major population

genetic changes induced by hunting pressure identified in other hunted species are reviewed. The advantages and disadvantages of non-invasive DNA sampling in addition with a description of the genetic markers used are also addressed. Finally, the hypotheses I aim to test in this research along with the outline of the thesis are specified.

1.2- The genus *papio*

Baboons [Primates; Cercopithecidae; genus *Papio*, Erxleben 1777, (Groves, 2001)] are a diverse and ecologically flexible group, distributed almost continuously across sub-Saharan Africa and the Arabian Peninsula. Traditionally, five parapatric “diagnosable entities” are described: Chacma baboons, Yellow baboons, Anubis baboons, Hamadryas baboons and Guinea baboons (Jolly, 1993; Nowak, 1999; Groves, 2001). These forms represent distinct morphotypes (e.g. pelage colour, presence of mane in adult males, skull size, Groves, 2001, Jolly, 1993), which are stable within local populations. However, in marginal populations, the effects of ongoing hybridization are detectable (Jolly, 1993). Some of these features are summarized in Table 1.1.

Baboon morphotypes are usually considered allotaxa since a geographic replacement between these forms occurs. The chacma baboon occupies the southern part of Africa, the yellow baboon is distributed mostly in eastern Africa, the guinea baboon is present in West Africa the north of equator, the anubis baboon is present in the savannah zone from Mali to Ethiopia and northern Tanzania and at several montane areas in the Sahara desert and the hamadryas baboon occupies Egypt and northern Somalia and the western Arabian Peninsula (Nowak, 1999) (see Fig. 1.1). Other forms, with apparent phenotypes, are sometimes grouped with Chacma baboons (Jolly, 1993). The Kinda (*P. h. kindae*, Zambia, Democratic Republic of Congo, and Angola) and the Ibean baboon (*P. h. ibeanus*, Kenya) are grouped with Yellow baboons (Jolly, 1993; Groves 2001). The Grayfooted baboon (*P. h. griseipes*, Zambia, Botswana, Zimbabwe, Mozambique, South Africa) and the Ruacana baboon (*P. h. ruacana*, Namibia, Angola) are grouped with chacma populations (Groves, 2001) (Fig. 1.1). The five most common morphotypes have been extensively studied for the last fifty years at several sites across sub Saharan Africa (see Baldwin and Teleki, 1972).

1.2.1 – Taxonomy, Phylogeny and Evolutionary History

Papio taxonomy has been strongly debated (Kamilar, 1996; Jolly, 1993; Groves, 2001), in particular, whether the five major recognizable forms should be considered full

species (*Papio ursinus*, *P. papio*, *P. anubis*, *P. cynocephalus*, *P. hamadryas*) (Groves, 2001) or as subspecies of *Papio hamadryas* (Jolly, 1993; Frost *et al.*, 2003; Kamilar, 2006) or even if hamadryas baboons should be regarded as a separate taxon (*Papio hamadryas*) from a the “savannah baboon” group (*Papio cynocephalus sp.*) (Smuts *et al.*, 1986). Here, I will follow Jolly’s (1993) argument that baboons are best defined as subspecies of *Papio hamadryas*, and I will refer to their vernacular names (Guinea, Anubis, Hamadryas, Yellow and Chacma) or by *P. h. papio*, *P. h. anubis*, *P. h. hamadryas*, *P. h. cynocephalus*, *P. h. ursinus*, respectively.

Table 1.1: Some of the external diagnostic features of the five forms within *Papio* genus (Groves 2001 and Jolly 1993).

Baboons subspecies	Pelage colour	Mane/Tail	Body colour and skull size
Chacma <i>Papio ursinus</i> (Kerr, 1792)	Black to brown or gray-buff dorsally	No mane Tail “broken”	Purple black face and male anal field, small around ischial callosities; Large skull
Yellow <i>Papio cynocephalus</i> (Linnaeus, 1766)	Dorsally yellow to yellow brown	No mane, Tail arched to more “broken”	Black face and bare areas around callosities; Medium-large skull
Anubis or Olive <i>Papio anubis</i> (Lesson, 1827)	Olive Brown	Large mane Tail “broken”	Purple-black facial colour and dark-gray to black around callosities; Medium large skull
Hamadryas <i>Papio hamadryas</i> (Linnaeus, 1758)	Silvery gray (males) and olive brown (females)	Huge mane Tail arched	Males with face and anal field bright pinkish red; females with gray face and skin not swollen around callosities; Medium size skull.
Guinea <i>Papio papio</i> (Desmarest, 1820)	Brownish red	Large mane; Tail arched	Face blackish red, pink male anal field, medium size skull.

There are a number of studies focusing on baboon systematic, either based on the variation in baboon facial patterns (for example Frost *et al.*, 2003) and molecular data (Lucotte, 1983; Williams-Blangero *et al.*, 1990; Newman *et al.*, 2004). These studies disagree in the taxonomic relationships within *Papio* possibly because: i) different molecular markers were used (blood proteins for Williams-Blangero *et al.*, 1990 and the “brown region” of mitochondrial DNA, Newman *et al.*, 2004), ii) lack of a broad sampling, iii) use of captive individuals with unknown (or imprecise) origin (Williams-Blangero *et al.*, 1990; Newman *et al.*, 2004) or iv) sampling in hybrid areas (Newman *et al.*, 2004). With the inclusion of new sampling sites, recent studies suggest that hybridization and genetic exchange may have

played an important function during the evolution of the genus (Burrell, 2008; Zinner *et al.*, 2009).

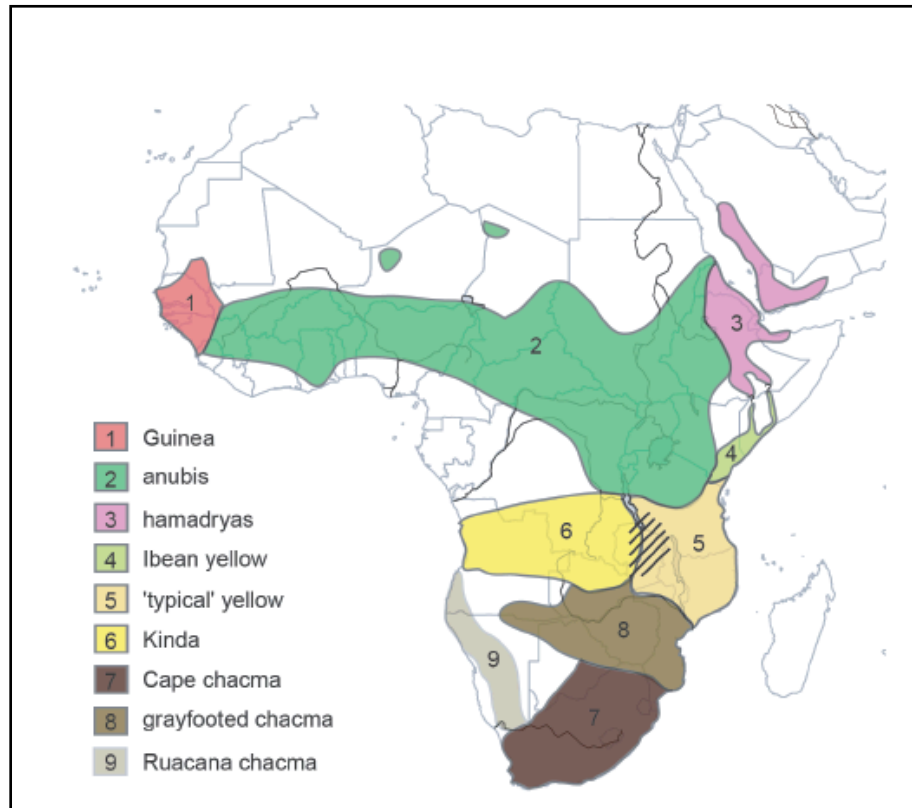


Figure 1.1 Distribution of *Papio* morphotypes (from Burrell, 2008)

In a study that included samples from West Africa baboons (Zinner *et al.*, 2009), eight well-supported haplogroups were detected, refuting the traditional classification of baboons into five taxa. Moreover, the deep divergences between haplogroups of Olive, Yellow and Chacma baboons and mixture of morphotypes within haplogroups, suggest paraphyly in the genus (op. cit.). Zinner *et al.*, (2009) proposes two forms of Chacma baboons, the Cape chacma (*P. h. ursinus*) and the Gray-footed (*P. h. griseipes*) but not the existence of *P. h. ruacana* as a taxonomic unit. It also suggests the separation of Kinda baboons (*P. h. kindae*) and the southern Yellow baboons as different units and proposes the existence of two well-supported taxonomic units of olive taxa in West Africa.

Molecular data suggest that the species ancestral to all extant *Papio* species inhabited southern Africa (Newman *et al.*, 2004; Lucotte, 1983) 1.75 MY ago. Newman *et al.* (2004) suggests that non-chacma subspecies would derive from an isolated northerly population of pre-Chacma baboons, which expanded to the north of the African continent. Guinea baboons, the next most divergent group, may have appeared after an east/west bifurcation, which, in

addition, led to the origin of Hamadryas / Olive / Yellow baboons (Newman, 2004). Zinner *et al.* (2009) suggest a more complex biogeographic history and argues several phases of population fragmentation, isolation, hybridization and introgression. They further suggest that the dispersal of baboon taxa from its basal branch in South Africa was hindered by cycles of expansion and retreat of savannah biomes throughout the late Pliocene and Pleistocene glacial and inter-glacial periods. Migration towards the north of the African continent might have occurred by a savannah corridor in east Africa (which is represented by pre-Yellow and pre-Hamadryas baboons) and a second through a savannah corridor in eastern Democratic Republic of Cameroon (corresponding to pre-Anubis and pre-Guinea baboons) (Zinner *et al.*, 2009).

This agrees with Jolly's "North-south split" scenario (Jolly 2009), although he only considered one south-north route. The north/south dichotomy found by Burrell (2008) leads Jolly (2009) to argue that an environmental barrier south of the equator in the early Pleistocene (probably a ring of evergreen forest occupying the southern mountain chain that expands from the Congo basin to Kenya/Tanzania coast) would stop baboons from reaching the northern woodland-savannah biomes. The permanent occupation of this forest by baboons might have been hindered by the presence of several forest-adapted monkeys and chimpanzee-like apes, thus acting as a barrier to their dispersal (Jolly, 2009). After the end of the forest barrier and with the drying climate, a continuous savannah corridor was opened: pre-Guinea and pre-Hamadryas baboons could then have encountered an ecologically favourable biome. Moreover, this area (that extends from the equator to the edge of the Sahara and from the Red Sea to the Upper Guinea) lacked competition with baboon-like species (Jolly, 2009), potentially allowing a fast expansion (hypothesized by Jolly as 10-15 Km every 6 years generation). Jolly (2009) argues that such event would also have had an impact in the social organization of these populations.

Regarding the origin of olive baboons presented by Jolly's hypothesis (Jolly 2009) Zinner *et al.* (2009) disagree since they found that, while Guinea baboons moved further west, pre-Olive baboons established relatively early in northern central Africa. Furthermore, it appears that Olive baboons have been fragmented into two (at least) allopatric populations, separated by the Niger River. A second expansion might have occurred, supported by a climatic change towards a more mesic condition to explain the hybridization between Olive and Guinea baboons in the west and Hamadryas and Yellow baboons in the east (Zinner *et al.*, 2009).

1.2.2 – Ecology

Baboons have adapted to a wide range of habitats across their distribution (Hall, 1968; Wolfheim, 1983; Jolly, 1993): they can be found in habitats from moist evergreen forest to desert, from seashore to mountain and from equatorial to subtropical regions. Their presence is limited by water and sleeping sites (Hamilton II, 1982; Gaynor, 1994), which can be tall trees (Anderson and McGrew, 1984; Schreier and Swedell, 2008), cliffs (Kummer, 1968), (Kansky, 2002), or caves (McGrew *et al.*, 2003; Barrett *et al.*, 2004). The use of several sleeping sites, a common situation (but see Kummer, 1968 or Dunbar and Nathan, 1972), is regarded as an advantage for the even use of their home range (Altmann and Altmann, 1970) or as a central place to forage (Gaynor, 1994). Although inhabiting significant distinct environments, the different subspecies studied so far (Chacma, Yellow, Anubis, Hamadryas and Guinea) are similar in their ecological niche space (Kamilar, 2006). It has been suggested that the great behavioural and life history flexibility showed by baboon populations is the result of selection in response to shifting environments (Alberts and Altmann, 2006). Baboons show a set of behavioural responses that enables ready adaptation to seasonal food availability (Dunbar, 1992; Barton *et al.*, 1996; Bronikowski and Altmann, 1996; Hill and Dunbar, 2002) and predation risk (Hamilton II, 1982; Cowlshaw, 1997a).

The subspecies within *Papio* are dietarily homogeneous (Dunbar, 1988). Baboons are able to adopt different dietary profiles, adapting their diet to seasonal changes by introducing new items. At the same time, they can be quite selective, using only a portion of the species available in their range or choosing only one or few parts of a food species (Whiten *et al.*, 1991). This highly selective generalist foraging strategy is thought to be the key of their evolutionary success (Alberts and Altmann, 2006).

Baboons use a particular home range, the size of which appears to be affected by the type of diet, the nutritional quality of the food resources, the foraging group size, the individual's body weight and the density of neighbouring troops (Dunbar, 1988) (see table 1.2). Seasonal changes of home range size and use, as well as, day journey length, are also related with changes in food availability.

Baboons show a decrease in the median size home range size and day journey length when grouped into habitat types of increasing food availability (Dunbar, 1988). Besides the change in group size, their behavioural flexibility allows them to shift their home ranges to areas of better food availability when food resources are scarce (Bronikowski and Altmann,

1996), to change foraging routes according to previous resource depletion (Gaynor, 1994) and even to reduce foraging effort (as measured by day journey length) as food availability increases (Barton *et al.*, 1992; Gaynor, 1994). This led Gaynor (1994) to classify the baboon's foraging strategy as time-minimising.

Table 1.2: Subspecies and number of population sampled; Social group size (mean of individuals), Range size (Km², mean), Day journey length (Km) and Range density (n/Km²) of 5 subspecies (from Dunbar, 1988)

Subspecies (n)	Size of social group	Range size	Day Journey Length	Range Density
Olive baboon (n=11)	41.2	9.6	3.14	4.3
Yellow baboon (n=4)	67.5	40.2	4.99	1.7
Chacma baboon (n=8)	48.0	15.1	5.68	3.2
Guinea baboon (n=1)	184.0	29.0	7.9	6.3
Hamadryas baboon (n=3)	68.5	21.5	9.4	3.2

Predation threats, along with food availability, play an important function in baboon's daily life. In some populations predation is responsible for the majority of deaths among adult females and juveniles (Cheney *et al.*, 2004; Cheney *et al.*, 2006). Natural primary predators of baboons are leopards (*Panthera pardus*), lions (*Panthera leo*) and hyenas (*Crocuta crocuta*) (Cowlshaw, 1994). Felid predators are limited to attack when visibility is limited and the most successful attacks occur at sleeping sites (Cowlshaw, 1994). Thus, baboons use cliffs, caves and taller trees as preferred sleeping sites in order to increase security against nocturnal predation (Hamilton II, 1982) although their choices may also be related to thermoregulation (Barrett *et al.*, 2004). In addition to food availability, home range use appears to be strongly influenced by the distance from the nearest sleeping site (Gaynor, 1994). Baboons prevent diurnal attacks by preferentially foraging in low-risk environments, despite the higher food availability in more dangerous habitats, and groom and rest in safe locations (Cowlshaw, 1997b). When refuges are available and widespread in their home range, they frequently tend to be close to them (in particular females of small groups) and pass very quickly through high-risk areas (Cowlshaw, 1997a). In the event of an attack, male baboons (but sometimes females, see Cheney *et al.*, 2003) give alarm barks, screams and threat vocalizations (Fischer *et al.*, 2001) and the group can chase, mob and attack predators (Cowlshaw, 1994).

1.2.3 – Social organization of Guinea baboons

Social organization is variable within the genus *Papio*. Chacma, Anubis and Yellow baboons live in groups formed by eight to 198 animals (usually between 30 to 60) (Nowak, 1999), with multiple individuals of either sex (but typically with more females than males) (Smuts *et al.*, 1986). Females are philopatric and form strong bonds with their relatives within the troop, while males disperse when they reach maturity. In these groups, matrilineal dominance hierarchies are formed and maternal kin occupy adjacent ranks (Smuts *et al.*, 1986). Gelada and hamadryas baboon societies are organized in a multi-level structure (Kummer, 1968; Kawai *et al.*, 1983; Swedell, 2002).

With respect to the social organization of the Guinea baboon, there is still a great debate in the literature (Bert *et al.*, 1967b cited in Boese, 1973 and Sharman, 1981; Dunbar and Nathan, 1972; Boese, 1973; Sharman, 1981) and although in recent years new data have come to light (Galat-Luong *et al.*, 2006; Maestripieri *et al.*, 2007; Patzelt *et al.*, 2011), there remains no consensus. My intention with the present section is to describe, in a chronological sequence, the results of key published work and to confront the respective conclusions.

Bert and co-authors (1967a and b, cited in Boese, 1973 and Sharman, 1981) studying Guinea baboons at sleeping sites in eastern Senegal described social groups as homogeneous bands without any sub-grouping. Although baboons were moving as a column, they displayed a disordered appearance (Bert *et al.*, 1967a and b, cited in Boese, 1973 and Sharman, 1981). Dunbar and Nathan (1972), on the other hand, observed some degree of structure inside troops, weak bonds between females and males and a relative autonomy of females.

Boese (1973) compared a captive social group (from Brookfield Zoo in Chicago) with free-ranging groups of Niokolo Koba National Park (Senegal). He concluded that Guinea baboons appeared to have a social organization intermediate between *P. h. hamadryas* and savannah baboons (*P. h. anubis*, *P. h. ursinus* and *P. h. cynocephalus*). Boese (1973) found free-ranging animals grouped in multi-male multi-female groups of 10 to 193 individuals (mean 84), which were usually divided into sub-groups (of approximately 10 individuals) for daytime foraging. The composition of a typical sub-group was of one adult male, three adult females, sub adult males, juveniles and infants. The sub-group separation varied with the type of habitat and time of day (Boese, 1973). Observations on captive individuals suggested that the sub-groups were the center of social and sexual activity and were usually organized around the adult male (Boese, 1973). He denoted a male-male bond and proposed two

hypotheses for the role of sub-adult males in the groups: i) assistance of the adult male in keeping females together and ii) protection of females when the adult male was absent. The sub-adult males benefited from this association by receiving support during conflict with other adult males and accessing young females for reproduction. However, sub-adult males still showed submission to adult male (Boese, 1973).

Sharman (1981) studied the ecology and social organization of free-ranging Guinea baboons at Mount Assirik (Senegal) and, although collecting data at approximately the same location as Boese (1973), reached slightly different conclusions. The main difference between the two studies is the variation in troop size (and perhaps dynamics) between wet and dry season, which was taken into account in Sharman's work. Sharman (1981) looked at the composition of foraging groups (i.e. a group of two to five baboons spatially separated from other baboons, moving between food sources), the age-sex association during progressions, inter-troop interactions and individual transfer between troops. He also examined the composition of social groups as a whole.

He concluded that the social organization and mating system of Guinea baboons resembled the other "savannah baboon" social organizations (Chacma, Yellow and Anubis), in which adults compete for mates. Since 15% to 30% of foraging groups studied contained adult females but not adult males, he found it to be highly improbable that adult males attempted to maintain control over a harem of adult females while the troop was foraging, in particular, under low visibility conditions (Sharman, 1981). In this study, adult males tended to be found together and frequently groomed one another, suggesting tolerance and affiliation. Since adult males do not seemed to compete for oestrus females, contrary to a harem society, and females showed frequent affiliative behaviour, he concluded that matrilineal kin groups associated with one or more adult males formed the social structure.

During the dry season, Sharman (1981) observed a fusion/fission dynamic. This author was unable to confirm if the membership of these subgroups was constant but fission-fusion of troops was harmonious, suggesting transference of individuals between troops. Indeed, Sharman (1981) observed the transfer of an adult male when two troops temporarily joined each other. He concluded that the fragmented social groups found in the dry season were a subset of the wet season troop, with closely associated social units staying together (Sharman, 1981).

Galat-Luong *et al.* (2006) collected data between 1975 and 2001, during the dry season, at the Niokolo Koba National Park. Their results describe a multi-level social structure similar to the one described to *Hamadryas* baboons by Kummer (1968) in which four hierarchical levels can be distinguished. The basic social unit was composed by eight to 10 individuals and were observed during the day in feeding, foraging and resting activities. When moving, each *one male unit* (OMU) was led by an adult male and were spatially distinct from other parties. OMUs joined into larger groups (5-65 individuals, mean 19 individuals) when moving. These groups could be distinguished spatially as they walked in long columns with similar groups, resembling Bert *et al.*'s observations (1967a and b, cited in Boese, 1973 and Sharman, 1981). A third level (22 to 249 individuals, mean of 62 individuals) formed the second level and a fourth level group was formed when at the sleeping site. The number of individuals within groups varied daily (Galat-Luong *et al.* 2006).

Galat-Luong *et al.* (2006) also observed high tolerance between males who controlled group movements (running to speed the progression of the group and shaking, jumping and prancing to change group movement). The authors conclude that the multi-level structure of Guinea baboon was not homologous to the one found in *Hamadryas* baboons since the fission and coordination of separate sub-groups was due to tolerance. Additionally, the affiliative behaviour between males and females allowed a greater degree of flexibility of movement (Galat-Luong *et al.*, 2006).

Maestripieri *et al.* (2007) reinvestigated Guinea baboon's Brookfield Zoo (Chicago) group, studied by Boese (1973) 34 years before. The results are consistent with Galat-Luong *et al.* (2006) in suggesting a harem-mating system. Females copulated with only one male of the sub-group and, similar to *Hamadryas* and *Gelada* baboons, these mating units corresponded to social units. The proximity between the adult male and female was associated with some social tension and agonism but the male coercion was not as intense as seen in *Hamadryas* baboon societies. Outside the harem, females interacted more with females, in particular with relatives, but had few or no interactions with non-member males (Maestripieri *et al.*, 2007). Most interestingly, the overall mating structure of this group is similar to the one observed by Boese (1973), suggesting stability in the social organization over time (Maestripieri *et al.*, 2007).

The disparity of conclusions between Boese (1973), Sharman's (1981) and the ones of Maestripieri *et al.* (2007) are probably linked with the limitations of captive populations.

Captivity can increase aggressive behaviour, strengthen dominance hierarchies and extend idiosyncratic behaviours (Galat-Luong *et al.*, 2006). This particular captive group is known to be inbred and presents low levels of genetic variability (Bruford and Altman, 1993; Alberts, 1999). Moreover, hybridization with *P. h. anubis* or *P. h. hamadryas* individuals might have occurred in the beginning of its colony history and their behaviour might not characterize the subspecies (Lacy and Foster, 1988). The differences in food availability between dry and wet season might account for the distinct conclusions found by Sarman (1981) and by Galat-Luong *et al.* (2006) since food availability (via climatic variables) is known to have great impact in social organization and dynamics of baboon groups (Dunbar, 1988).

Recently, Patzelt *et al.* (2011) reported lack of spatial pattern and variable composition in Guinea baboon social groups in Simenti (Niokolo Koba National Park, Senegal). After registering changes in composition of parties arriving in an open space, the authors (op. cit.) denoted weak troop cohesion, high variation in size and composition of parties, either daily or at a seasonal scale. Multi-male, multi-female units parties of 25 individuals were commonly observed but parties of less than 10 individuals, consisting of one adult male and several adult females were more frequent. The OMU was not attributed to be the basic unit of the social organization and the association in multi-males units was not stable over time. Additionally, Patzelt *et al.*, (2011) observed groups solely consisting of adult females and others formed by adult males only. It appears, therefore, that the social organization of Guinea baboons might not easily resemble savannah baboons or Hamadryas baboons, and perhaps represents a new social organization within the genus *Papio* (Patzelt *et al.*, 2011).

1.2.4 – Patterns of genetic diversity and population structure in baboons

Most studies of baboons estimating genetic diversity have used genetic data to investigate other aspects of baboon biology, such as phylogeny, social behaviour or demography (see review by Rogers, 2000). How variability is distributed in free-ranging populations has not been extensively investigated and since few researchers have used the same genetic markers, subspecific comparisons are difficult (Rogers, 2000).

Early studies on Anubis baboon populations, used variability in immunoglobulin allotypes (Olivier *et al.*, 1986) and showed considerable differentiation among localities separated by 50 to 100 Km. St. George *et al.* (1996) investigated the genetic variation at six microsatellite loci in Yellow baboons at two locations separated by 650 Km (Mikumi National Park in Tanzania and Tana River Primate Center in Kenya) and found substantial

genetic differentiation. The authors argued that genetic drift accounted for the differentiation between the populations (St. George *et al.*, 1996).

Rogers and Kidd (1993) studied four Yellow baboon social groups at Mikumi National Park (Tanzania) using restriction fragment length polymorphism of five loci nuclear markers. High variation was found (average proportion of polymorphic nucleotides of 1.0% to 1.3%) and the estimated effective population size was of 14,200 baboons. Their results also suggested that approximately two-thirds of the infants in the four social groups studied were fathered by males that dispersed less than 15-22 Km, while one-third of the infants are the progeny of males that have travelled farther. The authors concluded that the population behaves as a single genetic unit (Roger and Kidd, 1993; Roger and Kidd, 1996). Burrell (2008) found higher population structure when using mitochondrial than with microsatellites markers in Yellow, Kinda and Chacma baboon social groups. This evidence suggested female philopatry and male-mediated gene pattern for the three subspecies (Burrell, 2008).

A different pattern was found on the population genetic structure of Eritrean hamadryas baboons (Hapke *et al.*, 2001). The authors found two very different mitochondrial DNA lineages present at most sampling locations. The phylogenetic tree obtained, although structured, did not fit any geographic pattern and a greater proportion of genetic variation was found within subpopulations than among. They also found a positive correlation between the presence of shared haplotypes and geographic distance. Such pattern suggested female transfer between troops. Mitochondrial gene-flow was detected on a broader geographic scale (Hapke *et al.*, 2001). Hammond *et al.* (2006) confirmed such evidence in Saudi Arabian Hamadryas baboons by using sex-specific and bi-parentally inherited genetic markers. They found a four-fold greater structure for paternal than for autosomal and maternally inherited markers and a greater level of male relatedness within populations. They concluded that historical and instantaneous dispersal are female biased in hamadryas baboons. Female adoption or abduction by males from outside their natal group (Kummer, 1968) was highlighted as the most probable explanation for this dispersal pattern (Hammond *et al.*, 2006; Swedell *et al.*, 2010).

Hamadryas social organization and sex-biased dispersal pattern was associated with the very low levels of polymorphism on the Y chromosome of Saudi-Arabian Hamadryas baboons found by Lawson Handley *et al.* (2006). This effect did not seem related with a founder effect of the colonization of the Arabian Peninsula. The authors postulated that a high

discrepancy in reproductive success between the sexes and a sex ratio biased towards females have maintained the low effective population size of parental genes (Lawson Handley *et al.*, 2006).

Recently, Fickenscher (2010) found evidences for male philopatry and female-biased dispersal in Guinea baboons from Senegal. By using bi-parentally inherited genetic markers and a sex-determination marker, the author found higher population structure for males than for females and lower relatedness between males belonging to different troops. The population seemed to follow an isolation-by-distance pattern, with significant differentiation being obtained at 50 Km (Fickenscher, 2010).

1.2.5 - Threats affecting baboon populations

Most primate species are now facing serious threats to their existence, usually related to human population growth and activities (Di Fiore, 2004). Africa is of particular concern for global primate conservation. It includes 30% of all extant primates, often concentrated in relatively fragmented habitat and with relatively small geographic ranges (Chapman *et al.*, 2006) and most African countries have high human population growth rates¹. African human populations have many of the lowest incomes per capita in the world and therefore are highly dependent on natural resources¹. The human pressures on primate populations are varied and usually correlated and can be grouped in four main categories (Mittermeier, 1987; Chapman and Peres, 2001; Di Fiore, 2004; Chapman *et al.*, 2006):

- i) Habitat loss due to deforestation, land conversion, fragmentation or degradation;
- ii) Hunting as a source of food, for commerce, for traditional medicines or biomedical research, for adornments, bait or tourist souvenirs, and finally, when primates constitute an agricultural pest or are part of the trade in exotic animals;
- iii) Diseases and parasites transmitted by humans;
- iv) Climate change which can accelerate habitat loss and fragmentation or affect the phenological cycles of plant communities.

Baboons are not regarded by the IUCN as a priority of global primate conservation, being included in the category of Lower Risk (IUCN, 2010) and in annex II of CITES. On the one hand, baboons can persist in altered habitats (Wolfheim, 1983) due to high ecological

¹ <http://www.fao.org/>

adaptability and ability to adjust to new conditions (Hall, 1968). However, excessive habitat degradation or even total loss (frequently caused by agricultural practices), harvesting (when representing a source of food or for cultural, scientific and leisure activities) and direct persecution, can and do threaten baboon populations (Wolfheim, 1983; IUCN, 2010). Furthermore, this primate is perceived in many locations as causing the most damage to crops and is often regarded as a problematic resident of protected areas (Biquand *et al.*, 1992; Hill, 2000; Weladji and Tchamba, 2003). When compared with other raiding primate species, baboons feed on a wide variety of crops (Naughton-Treves *et al.*, 1998) and raid more often (Hill, 2000).

Several characteristics make baboons successful crop-raiders: intelligence, adaptability when facing new environments, a wide dietary range, opportunistic behaviour and complex social organization, which allows for co-operative actions (Maples *et al.*, 1976; Hill, 2000; Sillero-Zubiri and Switzer, 2001; Marais, 2006). The inclusion of provisioned food in baboon diets has a significant impact in their daily routine (Altmann and Muruthi, 1988) and is often preferred over wild food (Naughton-Treves *et al.*, 1998). Human food consumption by non-human primates translates into a foraging efficiency improvement: the metabolic demands are achieved sooner and provisioned food is more foreseeable (Saj *et al.*, 1999). As a consequence, in provisioned troops of baboons, the feeding periods, speed of travel, length of day-route and home range size is reduced, leading to dependence and regular use of this source of food (Altmann and Muruthi, 1988). The conflict between human activities and raiding baboons can ultimately lead to the removal or extinction of these populations (Wolfheim, 1983; Altmann and Muruthi, 1988; Biquand *et al.*, 1992; Nowak, 1999; Kansky, 2002). As a result, some populations throughout the baboon's range are at risk and pose conservation concern (Kansky, 2002; Weladji and Tchamba, 2003).

Hamadryas baboons have been exterminated in Egypt and reduced in numbers in some other areas within its range (Biquand, 1992; Nowak, 1999). Yellow baboons in the Masai-Amboseli Reserve have declined through a combination of natural and human-induced ecological factors (Wolfheim, 1983). Guinea baboons have declined in the Casamance region of Senegal because of excessive habitat degradation by agricultural practices and only a few fragmented populations persist (Wolfheim, 1983; Galat *et al.*, 1999-2000). One recent and well-described case is the Chacma baboon of the Cape Peninsula, South Africa. This population survives in natural areas, surrounded by human infrastructures and nine of the 10 remaining troops raid residents' habitations. As a consequence, adult males are actively

chased and killed by locals, which results in frequent take-over by other males and subsequently high rates of infanticide. This population was reduced to less than 250 mature individuals in 2002 (Kansky 2002).

Baboons are also frequently hunted when representing a relevant source of protein for locals (Fitzgibbon *et al.*, 1995; Fa *et al.*, 2005; Fusari and Carpaneto, 2006; Costa, 2010). Their ecological characteristics might increase their susceptibility to become a targeted species. Not only baboons are conspicuous to hunters, given that they live in large social groups and use loud vocalizations (Fa *et al.*, 2005) but also, their high abundance where other large animals have declined (e.g. Maisels *et al.*, 2001) can increase their hunting rate. Despite the lack of studies focussing on this problem from a conservation point of view, data suggest that some populations might be overharvested. For example, subsistence hunters in Arabuko-Sokoke forest (in Kenya) are recorded to have killed 683 Yellow baboons in one year (Fitzgibbon *et al.*, 1995).

If intense hunting pressure is combined with severe habitat loss and fragmentation, recolonization can be hindered (Novaro *et al.*, 2000), leading to the local extinction of such populations. Although baboons often do not seem as vulnerable as other species (when considering their ecological flexibility, abundance and large range size), the severity of anthropogenic pressures can potentially extinct the more threatened populations.

1.2.5.1 – The case study: Guinea baboons in Guinea-Bissau

The Guinea baboon (Fig. 1.2) is thought to constitute a threatened subspecies, although the lack of basic knowledge on their biology and social organization prevents an accurate evaluation of its threats. The conservation status of this primate was defined by IUCN (2010) as Lower Risk - Near Threatened (Oates *et al.*, 2008) and an assessment of the actual trend of its populations is lacking. The Guinea baboon has the smallest range of all baboon subspecies (Galat-Luong *et al.*, 2006) (Fig. 1.3). The populations have been decreasing in many locations and the subspecies suffered a significant range contraction (20-25%) over the last 30 years (Oates *et al.*, 2008). Habitat disturbance, excessive hunting and the capture and trade of juveniles have been identified as the main threats (Starin, 1989; Casanova and Sousa, 2007).



Figure 1.2: Guinea baboon in Guinea-Bissau (Photo by Joost Van Schijndel, CHIMBO)

In Guinea-Bissau, a small country located in the West African coast, Guinea baboon populations are reported to be declining due to intense human pressure (Gippoliti and Dell’Omo, 2003; Casanova and Sousa, 2007). Hunting for meat (Fig. 1.4) in combination with habitat loss appears to be the main threats affecting this population (Gippoliti and Dell’Omo, 2003; Casanova and Sousa, 2007). The decline of the Guinea-Bissau population probably started during the 1970’s (Cá, 2008), or 80’s (Casanova and Sousa, 2008) or even earlier, during the war of independence (1963-1974) (Ferreira da Silva, unpublished data). Baboons are believed to be still relatively common in the south of the country (Gippoliti and Dell’Omo, 2003) although residents claim to see baboons less frequently (Costa, 2010) and hunters state that baboons are becoming increasingly hard to find (Cá, 2008; Casanova and Sousa, 2007), in particular in the last two decades (Casanova and Sousa, 2007).

However, knowledge of the primate bushmeat trade is still incomplete. Hunting of primate species is illegal in Guinea-Bissau (DL n°21/1980) and locals are hesitant to provide information about this activity (Costa, 2010). Nevertheless, it is thought that large quantities of primates are hunted to supply a demand for bushmeat at the capital. The bushmeat trade occurs along the main connecting road to the capital, Bissau, although sometimes, bushmeat arrives by boat (Casanova and Sousa, 2007; Cá, 2008).

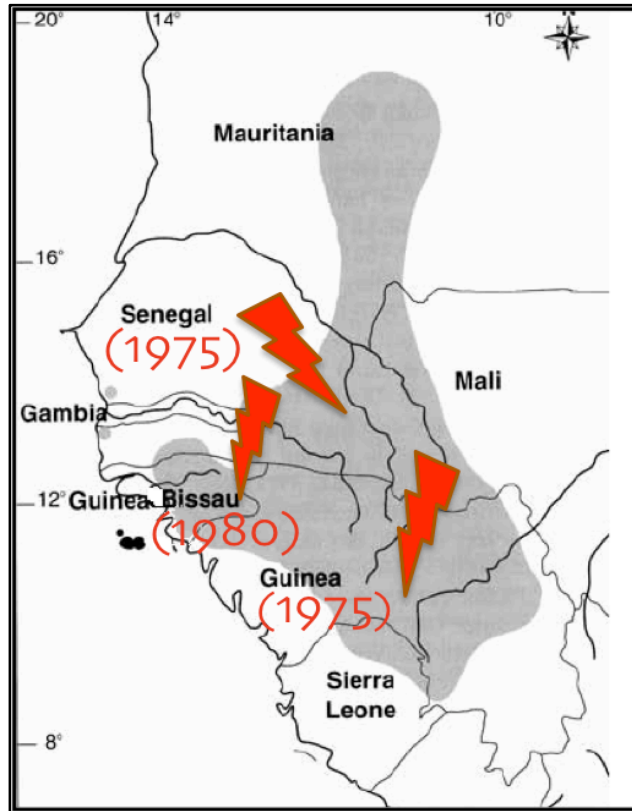


Figure 1.3: Guinea Baboon distribution range in West-Africa (adapted from Galat-Luong *et al.*, 2006) and locations where populations are thought to be declining (indicated by lighting bolt symbols). The year when populations started to decrease is indicated (Galat-Luong *et al.* 2006; Casanova and Sousa 2007).

Local individuals named *bideiras* (usually women) act as agents in the trade, ordering the bushmeat directly from the hunters and transporting it to Bissau (Cá 2008; Casanova and Sousa personal communication). Sometimes the hunters sell primate carcasses along the main road or directly to the urban markets (Cá 2008). In the capital, carcasses are sold in meat markets or to specialized restaurants that started to flourish during the 1980's (Casanova and Sousa, 2007; Starin, 2010). At the restaurants, primate meat is considered expensive, as four pieces of primate meat cost around 1,250 CFA (UD\$ 2.4) (Starin, 2010). In the south of the country, hunters sell baboons for around 5,000 CFA to the intermediary agent and show a stronger preference for hunting male baboons (Cá, 2008).

Additional factors contribute to the high mortality of this species in Guinea-Bissau. In the villages, baboons are also hunted as a result of crop-raiding conflicts and are consumed as a substitute of domestic meat (Costa, 2010). Probably as a result of hunting practices, it is also very common to observe young individuals being kept as pets (Casanova and Sousa, 2007) even within protected areas (Hockings and Sousa, 2011) (Fig. 1.5). Locals within Cantanhez National Park often refer to episodes where the military have hunted large quantities of

baboons to be consumed at their headquarters or as a replacement for their salaries (Casanova and Sousa, 2007). Skins of baboons, along with other mammals (Fig. 1.5) are traded at the capital for traditional medicine practices (Appendix 1).



Figure 1.4: A hunted baboon in Guinea-Bissau (found in Cufada Lagoons Natural Park). Photo by Cláudia Sousa

Hunting practices may also be changing behavioural patterns in Guinea-Bissau baboons. Hunting seems to take place during the night at sleeping sites and organized hunting parties have killed almost entire baboon groups in regions such as Quínara (Casanova, personal communication 2006) and Xitole (Cá, 2008). Contrary to other populations, which choose tall trees as their preferred sleeping sites (Anderson and McGrew, 1984), Guinea-Bissau baboons sleep in relatively shorter mangrove trees (but away from villages), possibly due to night attacks (Casanova, personal communication 2006). Additionally, in the south of the country, baboons feature greatly reduced vocalization when detecting human presence (Ferreira da Silva, personal observation), in contrast to the Senegalese population, where Guinea baboons are highly vocal (Byrne, 1981).



Figure 1.5: Pets and skins trade in Guinea-Bissau. Left: Guinea baboon kept as a pet in Cufada National Park. Right: Tradicional medicine market in Bissau, where at least seven species were morphologically identified [1 and 1a – Leopard (*Panthera pardus*), 2 – Chimpanzee (*Pan troglodytes* spp.), 3 – Crocodile (*Crocodylus niloticus*), 4 – Guinea baboon (*Papio hamadryas papio*), 5 – Putative lion (*Panthera leo*), 6 – Putative civete (*Civettictis civetta*), 7 – Antelope horns]

1.3 - Conservation Genetics and the importance of genetic diversity

Human impacts on wild populations, such as habitat fragmentation and hunting, can, in principle, lead to changes in genetic diversity (DiBattista, 2008). These anthropogenic threats reduce the size and/or increase isolation between demes, which expose the populations to random fluctuations. Such stochastic effects include environmental variation, demographic events and natural catastrophes and genetic processes (such as inbreeding and loss of genetic diversity through genetic drift) (Shaffer, 1981). The combination of such effects can accelerate extinction, even when the primary cause of the decline is unrelated (Frankham *et al.*, 2002). Gradually, with the reduction in population size and gene-flow, genetic drift (random changes in allele frequencies) may lead to fixation or loss of alleles (Lande, 1998) and to the accumulation of deleterious mutations in the population (Lynch *et al.*, 1995). With the loss of genetic diversity, small and isolated populations may have a compromised evolutionary capacity (Frankham, 2005). Their long-term adaptation to habitat instability or to future environmental changes (including diseases, parasites and predators) becomes limited (Frankham *et al.*, 2002).

Within the scope of *conservation genetics*, a discipline that aims to preserve and restore biodiversity by applying molecular genetic analyses (Frankham *et al.*, 2002), genetic diversity is central. The variety of alleles and genotypes present in a population is regarded as crucial to reduce the risk of extinction (Frankham, 2005). Although Lande (1988) argues that demographic factors could affect more rapidly than genetic factors the viability of wild populations (e.g. the “Lande scenario”), the interplay between genetic factors and other threatening processes can increase extinction risk (Frankham *et al.*, 2002).

Contemporary genetic diversity in a population can be affected by past and present population size changes, by natural selection, by mutation and gene flow. These factors also interact with spatial distribution and social structure (Frankham *et al.*, 2002), which act to further partition genetic diversity within populations. Small and isolated populations feature significantly reduced allelic diversity and heterozygosity, a smaller proportion of polymorphic loci (Amos and Harwood, 1998) and an altered allele distribution (Spencer *et al.*, 2000; England *et al.*, 2003). Demographic declines can vary in severity, being intense (when a population becomes small for brief periods) or diffuse (when a population gradually decreases over a longer period of time) (England *et al.*, 2003). Bottlenecks can therefore affect the indices of genetic diversity differently. Intense bottlenecks are thought to decrease allele diversity and loci polymorphism more severely than the diffuse ones (England *et al.*, 2003). Extinction of populations directly attributed to lack of genetic variation is rare (but see in cases of diseases, pests and parasites and in self incompatibility loci in plants, Frankham 2005 and Saccheri *et al.*, 2005). Inbreeding, on the other hand, seems to pose a more immediate risk to population persistence (Keller and Waller, 2002).

Inbreeding increases the probability that an individual is homozygous at a given locus. Naturally outbreeding populations contain deleterious alleles, which are usually recessive and persist at low frequencies. After a population decline or after increased isolation, individuals can be more likely to breed with related individuals, increasing the chance of expressing deleterious recessive alleles (Frankham 2002). This phenomenon, which leads to diminished viability of populations and to the reduction of the individuals’ reproductive fitness (Frankham 2002) has been described as *inbreeding depression*. Species with lower intrinsic growth rates are affected more severely because the recovery from demographic bottlenecks can be slow and the population fitness can be reduced through these processes (e.g. biased birth sex ratio) (Mills and Smouse, 1994).

Nevertheless, inbreeding may have a minimal impact on population fitness if selection acts against inbred individuals. While expressing deleterious recessive alleles, those individuals can be purged from the population, which reduces genetic load (Keller and Waller, 2002). DONE was explained by the loss of harmful alleles, which *per se* increased the resistance to inbreeding (Groombridge *et al.*, 2000; Hoglund, 2009). However, such purging will not be efficient against mildly deleterious mutations that constitute a great part of the genetic load (Keller and Waller, 2002). Also, in addition to other demographic scenarios, if inbreeding is fast and concurrent with a significant reduction of the population effective size and if the population is not completely isolated, the deleterious genetic load might not be efficiently eradicated by natural selection (Keller and Waller, 2002). The effects of human disturbance on genetic variation in wild populations are, however, not clear. DiBattista (2008), reviewing the evidence, found that although habitat fragmentation significantly decreased genetic variation, hunting had no noticeable effect. In the next section, I review the populations hunting-induced genetic changes described so far.

1.3.1 – Genetic changes induced by hunting pressure

The genetic effects of hunting pressure (i.e. hunting rate) on populations are less noticeable than the demographic threats of over-harvesting (i.e. hunting over a sustainable rate, compromising the population persistence) and to date have been mainly investigated in freshwater fishes species, ungulate species, carnivores and elephants (*Loxodonta africana*) (Harris *et al.*, 2002; Allendorf *et al.*, 2008; Allendorf and Hard, 2009). Hunting pressure can potentially precipitate changes in the population structure leading to genetic changes (Harris *et al.*, 2002; Allendorf *et al.*, 2008; Allendorf and Hard, 2009). Three types of genetic change are predicted: i) changes in population structure and gene-flow between demes, ii) loss of variation and iii) allele frequency changes due to selection (Harris *et al.*, 2002; Allendorf *et al.*, 2008).

In genetically structured species, heavily hunted sub-populations can suffer a reduction in their size or density or even total extirpation. This is expected to lead to a change in gene-flow between the subpopulations. If gene-flow is decreased and those populations became more isolated, genetic drift and loss of genetic variation can increase. If, on the other hand, an increase in the number of migrants occurs, genetic swamping with attendant loss of local adaptation can be precipitated. Loss of local adaptations in particular, can be accelerated

if differential harvesting within populations exists and the less resilient and productive individuals disappear, leading to loss of fitness (Allendorf *et al.*, 2008).

If harvesting targets specific sex or age classes, it may affect the population's demographic structure, leading to a reduction in the effective population size. Removal of certain individuals has the potential to impose artificial selection and an evolutionary response. The latter occurs if the selection is towards heritable phenotypic characters (e.g. minimum body size, horn length or antler size) (Coltman, 2008). A reduction in effective population size will usually lead to a loss of genetic variation (Allendorf *et al.*, 2008). Ryman *et al.* (1981) simulated moose and white-tailed deer populations with relatively smaller effective sizes and subjected to different hunting regimes. The authors found that hunting regimes targeted at younger animals would increase generation intervals, leading to a loss of genetic variation in few generations. Hunting regimes targeting males can affect heterozygosity and the male effective population size. Nevertheless, this might have a limited effect in allelic diversity as this measure is mostly affected by decreases in census population sizes (Allendorf *et al.*, 2008).

Hunting can also disrupt the structure of breeding groups in social species and stimulate long-term genetic changes (Cowlshaw and Dunbar, 2000). The removal of certain individuals can induce a change of gene flow between social groups (see review by Harris *et al.* 2002). In species with male-biased dispersal patterns, hunting that targets males can decrease gene flow between groups, leading to a greater genetic differentiation between demes. Ellsworth *et al.* (1994), for example, found a higher level of mtDNA structure in hunted populations of white-tailed deer when compared with other populations, a result attributed to males harvesting practices.

The hunting of adult males can also have a direct consequence on the increase of infanticide rate and, consequently, on the growth of the population. Swenson *et al.* (1997) found that the mortality of adult brown bear males was correlated with cub survival, which was significantly lower up to 1.5 years in a heavily hunted area when compared with areas with no hunting. Also, by changing the sex ratio, mate choice may become less specific (Jachmann *et al.*, 1995). The restriction of the mating selection could reduce the genetic quality of descendants (Neff and Pitcher, 2005) or lead to inbreeding depression if a sudden reduction in the level of heterozygosity occurs (Nyakaana *et al.*, 2001).

Group size can change in response to hunting pressure (but see Croes *et al.*, 2006 where no differences were found in primate group size between hunted/non-hunted areas). On the one hand, groups can become smaller due higher mortality rate (e.g. himalyan thar, *Hemitragus jemlahicus* in Carneys Creek, Tustin and Challies, 1978). On the other hand, groups can become larger to reduce the risk of attack by hunters (Cowlshaw and Dunbar, 2000; Strier, 2007). Increased interspecific association rates between red colobus (*Procolobus badius*) and Diana monkeys (*Cercopithecus diana*) in Taï National Park, Ivory Coast, seem to be related to the chimpanzee hunting season (Noe and Bshary, 1997). Nyakaana *et al.* (2001) found that in elephants, the stress posed by hunting pressure induced different hunted family groups to fuse, which changed the genetic pattern of social groups (Nyakaana *et al.*, 2001). Jedrzejewski *et al.* (2005) also found different genetic patterns in breeding pairs of wolves (*Canis lupus*) in Belorussia. Within this severely hunted population, wolf breeding pairs were formed by unrelated individuals or by half-sibs (Jedrzejewski *et al.*, 2005). The resident packs accepted these apparently long-distance immigrants as a means to nurture young individuals (Jedrzejewski *et al.*, 2005). In this case, however, the authors found high levels of heterozygosity in the population due to immigration of individuals from less hunted areas.

The effects of harvesting can have broader spatial amplitude than initially expected. In environments where hunting practices are frequent, for example in areas more accessible to hunters (on the periphery of the forest, Fitzgibbon *et al.* 1995 or in small fragments of forest, Peres, 2000), localized demographic declines can precipitate a “sink” effect (Pulliam, 1988; Novaro *et al.*, 2000). Dispersing animals will be attracted to those areas because the habitat remains unaffected and the high mortality rate is difficult to detect (Delibes *et al.*, 2001). For example, Robinson and co-authors (2008) found increased immigration of younger cougars (*Puma concolor*) to supervised game areas. In this case, the immigrants compensated for the mortality due to hunting and density remained unchanged (Robinson *et al.* 2008). They concluded that the sink effect could easily conceal a hunting-induced population decline (Robinson *et al.*, 2008). Hunting practices targeting male lions in western Zimbabwe (Loveridge *et al.* 2007) biased the sex ratio towards females and induced a “vacuum effect”. Male immigrants occupied the male depleted home ranges and as a result, the infanticide rate increased (Loveridge *et al.*, 2007).

Although there are some examples of changes in group structure and genetic diversity induced by hunting (Tustin and Challies, 1978; Ryman *et al.*, 1981; Standford 1995; Nyakaana *et al.* 2001), habitat disturbance or population density can act as confounding

variables, preventing a straightforward relationship (Cowlshaw and Dunbar 2000; DiBattista, 2008). Also, high immigration rates from adjacent populations can artificially increase genetic diversity in the population (e.g. Jedrzejewski *et al.*, 2005), preventing a correct evaluation of the threat those populations are facing. Therefore, the correct assessment of the population structure is required (Harris *et al.* 2002).

1.3.2 - *The use of non invasive samples in the study of wild primates*

A variety of DNA sources, including blood, tissues, hairs, faeces, urine, semen, buccal swabs and masticated wedges have been used for genetic analyses of wild primates. However, blood and tissue are difficult to sample and these practices are considered unethical. The collection of blood usually involves the immobilization and anaesthesia of the animal. Therefore, collectors must receive professional and/or veterinary training in order to take blood without injuring the animal (Woodruff, 1993; Di Fiore and Gagneux, 2007). In addition, the capture of primates can be logistically demanding or even impossible (especially if a large number of samples are required) and carries risks to the animal and researcher's health (Piggott and Taylor, 2003).

Since the use of the *Polymerase chain reaction* (PCR, Saiki *et al.* 1985) became common, non-invasive DNA sampling has been frequently used in primate studies (Woodruff, 1993). The first applications to non-human primates started with the discovery that a single human hair root contained sufficient DNA (von Beroldingen *et al.*, 1987). Using plucked hair from several non-human primates [chimpanzees, gorilla (*Gorilla gorilla*), orang-utan (*Pongo pymaeus*), lion-tailed macaques (*Macaca silenus*), gibbons (*Hylobates*) and rhesus macaques (*Macaca mulatta*)], Morin *et al.* (2001) were able to amplify sequences of mitochondrial DNA genes and microsatellite loci. In every case studied, it was confirmed identical quality in the sequences and microsatellites patterns to DNA derived from hair and other tissues (blood and placenta) (see Woodruff, 1993 for a review of the first applications). Of all non-invasive DNA sources, faeces have the greatest potential for field sampling (Morin and Woodruff, 1996) and have been widely used (Fernando *et al.*, 2003). Animals defecate regularly, so faeces can be easy to find and the collection, storage and transport requires little technology or expense (Fernando *et al.*, 2003). DNA purification from faeces is feasible given that there are host cells on the surface, discarded from the intestinal lining during defecation (Kohn and Wayne, 1997). However, the faecal DNA is in low quantity and quality and contains a mixture of microorganisms, undigested food, digestive enzymes, mucus, bile salts,

and bilirubin (Sidransky *et al.*, 1992), potentially capable of inhibiting the PCR reaction (Kohn and Wayne, 1997; Beja-Pereira *et al.*, 2009).

Contamination with high-quality DNA from other sources is another drawback of using this source of DNA. Due to the severity of this contamination, extra precautions are required during DNA extraction (such as working in a separate laboratory facility, away from where high quality DNA is handled or stored) or during PCR. This can increase the cost and time of laboratorial procedures (Piggott and Taylor, 2003). Additionally, the low quantity and DNA degradation can lead to errors (as false alleles or allelic dropouts Taberlet *et al.*, 1996) and even non-amplification (Kohn and Wayne, 1997).

The protocol for collection and storage of faecal samples for molecular analysis must ensure that the quantity and quality of DNA recovered is maximised (Piggott and Taylor, 2003). Faeces should be collected as fresh as possible to avoid bacterial activity (Fernando *et al.*, 2003). During sample storage, the molecular environment of the DNA should be hostile to enzymatic activity, which can be achieved by physical or chemical means (Piggott and Taylor, 2003; Beja-Pereira *et al.*, 2009). If the species is found in remote locations or sampling requires many weeks, an adequate storage protocol should be employed (Piggott and Taylor, 2003).

Faecal DNA quality, extraction and amplification success varies with the storage solution, the extraction method and with the species (Whittier *et al.*, 1999). Environmental variables such as the temperature and humidity at time of collection also influence the success of such techniques (Nsubuga *et al.*, 2004). The factors affecting DNA quantity in faecal samples and the best preservation and extraction methods have been investigated thoroughly (Frantzen *et al.*, 1998; Flagstad *et al.*, 1999; Whittier *et al.*, 1999; Piggott and Taylor, 2003; Nsubuga *et al.*, 2004; Roeder *et al.*, 2004). More recently, Roeder *et al.* (2004) described a method named “two-step” in which samples are soaked in 99% ethanol for one day and then desiccated in silica beads. This preservation method recovered two to 2.5 times more DNA than the ethanol and silica methods, respectively (Roeder *et al.*, 2004) and has been commonly used.

1.3.2.1 - Molecular Markers used in non-invasive DNA samples

1.3.2.1.1 – Microsatellites loci

Microsatellites (SSR – simple sequence repeat or STR- short tandem repeat) are regions of the genome comprising variable numbers of tandem repeats of a 1 to 10 base-pair nucleotide motifs (Di Fiore, 2003). This marker has been frequently used in the last decade to access nuclear DNA variation at different genetic levels: assessment of parentage and individual identity, estimation of relatedness between pairs of individuals and populations, evaluation of population structure and dispersal patterns and in evolutionary studies of related species (Clisson, 2000; Di Fiore, 2003). Microsatellites are often used in population-level studies because: i) they are randomly distributed throughout the genome, commonly in non-coding regions and are usually selectively neutral; ii) are hypervariable within populations, showing higher mutation rates than other nuclear regions (Weber and Wong, 1993); iii) have co-dominant inheritance, which allows direct scoring and iv) their analysis only requires miniscule amounts of template DNA present in non-invasive DNA samples (Di Fiore, 2003). In addition and due to its high mutational rate, microsatellites are often the genetic marker of choice for the detection of demographic bottlenecks in natural populations (Cornuet and Luikart, 1996; Beaumont, 1999; Luikart *et al.*, 1999; Spencer *et al.*, 2000a; Garza and Williamson, 2001).

However, the low amounts of DNA in non-invasive samples make microsatellite analysis error-prone (Taberlet *et al.*, 1996). Two main types of scoring errors are commonly experienced: allelic dropout (the stochastic failure of the amplification of one allele) and false alleles (the production of amplification artefacts) (Piggott and Taylor, 2003; Di Fiore, 2003). Null alleles, on the other hand, are allelic variants that do not amplify due to mutations in one or both of the primer binding sites (Pemberton *et al.*, 1995). Although locus dependent and not particularly related with non-invasive DNA samples, its occurrence can introduce errors into population allele frequency and coefficients of pairwise relatedness (Di Fiore, 2003).

Inbreeding and population structure analysis can be severely affected by allelic dropout since heterozygotes are mistakenly identified as homozygotes (Taberlet *et al.*, 1999). In particular, the assessment of relatedness and kinship or individual assessment will be severely affected. One famous example is the different conclusions regarding extra-community mating in chimpanzees' populations reached by Constable *et al.* (2001) and Vigilant *et al.* (2001) due to genotyping errors.

The genotyping error rate in non-invasive DNA samples depends on a wide range of biological, technical and human-related factors (Piggott and Taylor, 2003; Bonin *et al.*, 2004). Biological factors include the type of sample (faecal samples usually produce higher genotype errors than pulled hair samples), inter-individual differences (Goossens *et al.*, 2000) and season or age related factors (seasonal dietary and/or climatic characteristics; Piggott and Taylor, 2003). Usually, if the amount of DNA is diminished, the rate of genotyping error is increased. Technical factors include stochastic effects of DNA template sampling or preferential amplification of one of the alleles: in a very diluted DNA extract, sometimes only one of the two alleles is pipetted, amplified and detected (Taberlet *et al.*, 1996). In non-invasive DNA samples of baboons, elephants and chimpanzees, Buchan *et al.* (2005) found that median allele size had a significant effect on amplification success. Smaller loci have higher amplification success and a decrease of 12-15% for each 100 base pair increase in allele size amplified can be expected (Buchan *et al.*, 2005). Nevertheless, human factors (as sample swapping, pipetting errors or confusion on data entry) should not be overlooked (Bonin *et al.*, 2004).

Guidelines to detect genotyping errors have been developed (Navidi *et al.*, 1992; Taberlet *et al.*, 1996; Frantz *et al.*, 2003). Navidi *et al.* (1992) developed a “multi-tubes approach”, which involves dividing the sample among several tubes, and then amplifying and typing each tube separately. Taberlet *et al.* (1996) further determined the number of repetitions necessary to obtain a reliable genotyping with a confidence level of 99%. However, these approaches have been criticized since conducting multiple PCRs is expensive, time and sample consuming, in particular when dealing with non-invasive DNA samples, which usually have low volume and yield of extracted DNA (Waits and Paetkau, 2005). Another approach is to pre-screen the samples in order to detect the most error-prone ones (e.g. with lower amount of DNA) and eliminate them from the following analyses (e.g. Morin *et al.* 2001).

1.3.2.1.2 – Mitochondrial DNA

Mitochondrial DNA (mtDNA) is a small, covalently closed circular molecule, about 16 to 20 kilobases long. It has 36 to 37 genes and a control region that includes the “D” or displacement loop, which exercises control over mtDNA replication and RNA transcription (Awise *et al.*, 1987). This molecular marker evolves faster than the nuclear DNA (5-10 times faster, Wilson *et al.*, 1985). Most genetic changes consist of simple base substitutions and

indels (addition/deletion), which can be one or few nucleotides or large length differences (Awise *et al.*, 1987). However, the rate of evolution differs for regions of the molecule, which allows its use for a variety of questions (Wan *et al.*, 2004). Mitochondrial DNA is usually considered non-recombining (from which it can be assumed that the entire molecule has the same evolutionary history, but see Rokas *et al.*, 2003), is commonly considered a neutral marker (but see Ballard and Rand, 2005) and is inherited strictly along the maternal line (which theoretically provides information regarding maternal relatedness and sex-specific population structure) (Di Fiore, 2003; Ballard and Whitlock, 2004; Ballard and Rand, 2005; Hurst and Jiggins, 2005).

Some of the commonly accepted mtDNA features have been challenged for animal species (reviewed by Rokas *et al.*, 2003; Slate and Gemmell, 2004). At least two phenomena contradict an inflexible maternal inheritance: i) paternal leakage, which was observed in a wide variety of animal species (including humans) and ii) double uniparent inheritance (i.e. while female offsprings receive their mother's mtDNA, male decedents inherit both the father's and mother's mtDNA and become heteroplasmic), demonstrated in bivalve species (Rokas *et al.*, 2003; Slate and Gemmell, 2004). Additionally, strict mtDNA non-recombination has been contested by the presence of recombination enzymes in animals' mitochondria and by experimental evidences in wild populations of various taxonomic groups that proved recombination (Slate and Gemmell, 2004). Although animal mtDNA recombination is difficult to detect, its presence can impact molecular evolution research (Rokas *et al.*, 2003; Zsurka, 2007). For example in phylogenetic reconstruction studies, the shape and topology of the phylogeny tree may be affected (Rokas *et al.*, 2003; Zsurka 2007). In such cases, the time to the most common ancestor of the sequences will be most probably underestimated and the molecular clock may be incorrectly rejected (Rokas *et al.*, 2003).

In the case of non-invasive DNA samples, mtDNA has an important advantage when compared with nuclear DNA. In one cell there are hundreds of copies of mtDNA while there are only two copies of nuclear DNA, which allows a higher amplification rate in these types of samples (Kohn and Wayne, 1997; Frantzen *et al.*, 1998; Poole *et al.*, 2001; Lucchini *et al.*, 2002; Waits and Paetkau, 2005).

1.4 – Aims and Hypotheses

The overarching aim of this thesis is to investigate the effects of hunting practices on the genetic diversity and demographic structure in a baboon population. Focusing in the Guinea baboon in southern Guinea-Bissau, this PhD has three main goals:

- i) To investigate the patterns of past and current hunting practices;
- ii) To investigate the effect of hunting pressure on genetic diversity of a hunted primate species;
- iii) To investigate the possible changes in the population structure, dispersal strategies and genetic patterns of social units caused by hunting practices.

Additionally, I aim to test the following hypotheses:

1) The Guinea-Bissau baboon population has undergone recent changes in its population structure and show reduced genetic diversity

A possible effect of hunting practices on the populations is the restriction of gene-flow between sub-populations (Harris *et al.*, 2002; Allendorf *et al.*, 2008; Allendorf and Hard, 2009), which can lead to lower levels of genetic diversity and higher risk of population extinction. As Guinea baboons in GB have been heavily hunted in the last decades, I expect sub-populations to be demographically isolated and as a result, to have reduced genetic diversity.

To address this hypothesis, I will use two genetic markers (microsatellite loci and mtDNA) and will: 1) assess the genetic diversity within and among social groups using non-invasive DNA samples; 2) compare the genetic diversity levels with other baboon populations subject to lower hunting pressure. To assess the effects of hunting on the population structure, I will 3) compare the pattern of spatial genetic differentiation between different genetic markers, for which the mode of inheritance differs. As the dispersal strategy in Guinea baboons seems to be female-mediated (Fickenscher, 2010), I anticipate a pattern of historical female-biased dispersal pattern in GB: lack of isolation-by-distance and spatial structure for the distribution of mtDNA haplotypes (similarly to what was found in *Hamadryas* baboons by Hapke *et al.*, 2001). Nevertheless, hunting-driven restriction in gene-flow may create spatial genetic discontinuities located concordantly with human settlements or roads (a proxy for hunting pressure) for both genetic markers (Manel *et al.*, 2003).

2) Hunting pressure will affect dispersal strategies in GB population

Intense hunting pressure has the potential to change i) the individuals behavioural patterns (e.g. inducing secretive behaviours, Cowlshaw and Dunbar, 2000); ii) the social groups composition (e.g. sex ratio and age structure, Cowlshaw and Dunbar, 2000; Loveridge *et al.*, 2007) and/or iii) the groups number or density across space (Watanabe, 1981; Rosenbaum *et al.*, 1998; Nijman, 2004; Allendorf *et al.*, 2008; Kuehl *et al.*, 2009). These changes seem to influence condition-dependent dispersal strategies in hunted species (Loveridge *et al.*, 2007; Costello *et al.*, 2008; Pérez-González and Carranza, 2009; Pérez-Espona *et al.*, 2010). A possible response of primate populations is to alter the dispersal rates of individuals usually remaining philopatric (Isbell and Vuren, 1996; e.g. Sugiyama, 1999) and as a result, the species-specific dispersal patterns (usually biased towards one sex) can be reversed or become less intense (Strier, 2007). Therefore, I predict hunted Guinea baboon populations will display a disrupted sex-biased dispersal pattern.

To test this hypothesis I will: 1) assess differences between males and females in instantaneous dispersal patterns using bi-parentally inherited markers, such as microsatellite loci (Goudet *et al.*, 2002; Prugnolle and de Meeus, 2007); 2) compare the sex-biased dispersal patterns between Guinea baboons inhabiting Guinea-Bissau and Senegal, a population subject to lower human pressure (Galat-Luong *et al.*, 2006; Fickenscher, personal observation).

3) Hunting pressure will affect relatedness and sex ratio in social groups

A behavioural response to high hunting rates is the amalgamation of unrelated conspecifics in social groups (e.g. Noe and Bshary, 1997; Jedrzejewski *et al.*, 2005; Gobush *et al.*, 2009). Groups may become larger to reduce the risk of attack by hunters (Cowlshaw and Dunbar, 2000; Strier, 2007), either by including several hunted family groups (Gobush *et al.*, 2009) or long-distanced immigrants (Jedrzejewski *et al.*, 2005). This defensive behavior changes the genetic pattern of social groups that, in average, display lower levels of relatedness. Moreover, harvesting can change the genetic pattern of social groups if specific sex classes are targeted. In this case, by increasing mortality towards a specific sex, the social group's sex ratio can become significantly biased, which has important consequences in the dispersal strategies patterns or in the population growth rate (e.g. Swenson *et al.*, 1997; Loveridge *et al.*, 2007).

As hunting practices in GB induced behavioural modifications in Guinea baboons (such as the choice to sleep in mangrove trees or reduced vocalization when human presence is detected, Ferreira da Silva, personal observation, Casanova, personal communication 2006), I expect other type of modifications in the population social dynamics. Therefore, I expect to find disrupted social groups, possibly formed by unrelated individuals (average within group relatedness of zero).

Additionally, there is evidences to suggest a preference for males by hunters (Cá, 2008). However, the geographically widespread observation of baboons being kept as pets (Casanova and Sousa, 2007; Hockings and Sousa, 2011) suggests mortality of both lactating females and adult males. Therefore, I expect to find a biased sex ratio composition in social groups.

To test the hypothesis, I will: 1) estimate mean group relatedness; 2) determine the sex of individuals by using a molecular determination protocol and estimate sex ratio of social units; 4) compare between the genetic pattern of GB and Senegalese social units.

1.5 - Thesis Outline

To accomplish the project aims, I collected non-invasive DNA samples in southern Guinea-Bissau from three different locations and used two genetic markers (mtDNA and microsatellite loci). Bushmeat tissue samples, collected at the urban markets, were also analysed. In Chapter 2, sampling sites and laboratory procedures are described. In Chapter 3, the characterization of current and past hunting practices towards Guinea baboons, including an estimation of the quantity of baboon's traded at urban markets, are presented. In Chapter 4, I investigate the population structure and genetic diversity of the southern Guinea-Bissau baboons. To test if human settlements can represent a barrier to dispersal, I used a landscape genetic approach (Manel *et al.*, 2003) to determine the location of genetic discontinuities and compare the pattern of population differentiation between the genetic markers. In Chapter 5, the consequences of hunting pressure on the sex ratio and relatedness within social units are investigated. By comparing the southern Guinea-Bissau population with a non-hunted one (inhabiting the Niokolo Koba National Park, in Senegal), the effects of harvesting on the sex-biased dispersal patterns are investigated. Chapter 6 presents a summary of the main findings of this study and the implications for the conservation of Guinea Baboon population in

Chapter 1: Introduction

Guinea-Bissau. Each chapter is self-contained and presents its own introduction and objectives, methodology, results, discussion and references used.

1.6 - References

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Chapter 2 General Methodology

Galinha pindradu ka ta sibi si kaminhu lundju

(The chicken that travels held by the legs does not know how long the way is -

Guinea-Bissau popular saying suggesting that he/she that does not walk cannot understand
how long it is)

This general methods chapter describes the procedures common to the molecular analyses performed in the following chapters. It covers a description of the sampling sites and strategy, the laboratory methods (including the DNA extraction, genetic markers and DNA amplification conditions), genotyping procedures and data quality assessment. It ends with a description of the genetic diversity uncovered by the genetic markers adopted and with a critical discussion of the extraction/amplification success.

2.1 - DNA Sampling

2.1.1 – Study Area – Guinea-Bissau

Guinea-Bissau is a small country (36,125 Km² surface area) located in West Africa (10°59' - 12°20'N and 13°40' - 16°43'W). It is bordered to the north by Senegal, to the east and south by the Republic of Guinea and to the West by the Atlantic Ocean and it comprises a continental mainland and an archipelago, the Bijagós (Fig. 2.1).

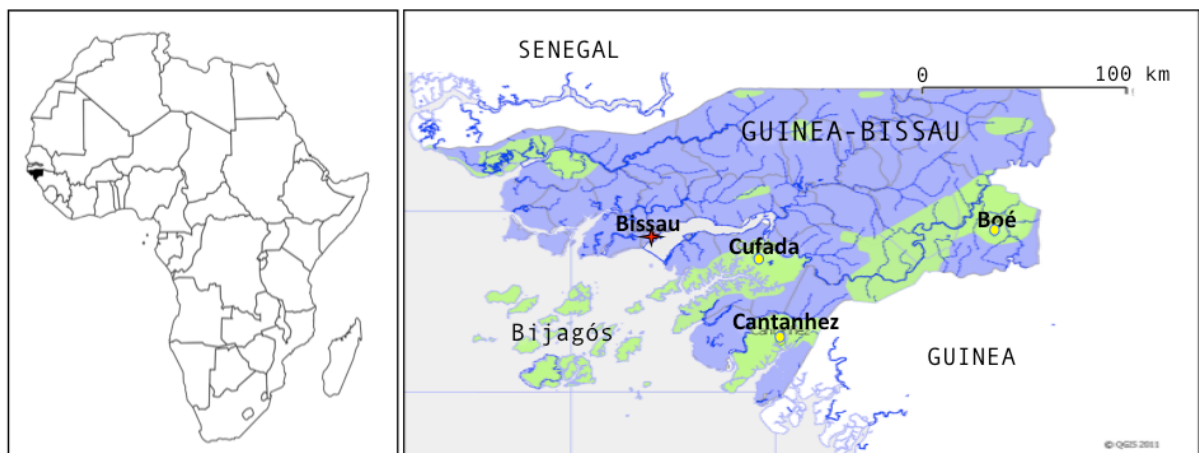


Figure 2.1 Geographic location of Guinea-Bissau in the African continent and of sampling locations (Cantanhez, Cufada and Boé).

Guinea-Bissau was a Portuguese colony between its “discovery” by Nuno Tristão in 1446 until 24th September 1973. This date marks the unilateral proclamation of the independence of the Republic of Guinea-Bissau by the PAIGC party (African Party for the independence of Guinea and Cape Verde) and it was a consequence of a large-scale guerrilla war against the Portuguese forces. The recent political history of Guinea-Bissau is marked by several military coups and political instability. The civil war that started in May 1998 and

lasted for 11th months destroyed most of the infrastructures in the country and resulted in the displacement of thousands of persons².

Guinea-Bissau is one of the world's poorest countries. More than 50% of the population lives below the poverty line, there is a high level of inequality of income distribution and life expectancy at birth is only of 43 years. The country's estimated population in 2001 was 1.3 million, with a growth rate of 2.23% (World Bank, 2004). The primary sector (agriculture, livestock and fishing) is the greatest contributor to the economy (PNUD, 2006). The two main sources of legal income into the country are cashew nuts and fishing licenses (Anonymous, 2002).

The country's rainfall patterns are conditioned by the seasonal migration of the intertropical convergence zone (ITCZ) (Fosberg *et al.*, 1961). The rainy season begins in early June and ends in early November, when the ITCZ migrates northwards and to the Gulf of Guinea, respectively. Annual rainfall is geographically highly variable, ranging from 2,400 and 2,600 mm in the southwest to 1,200 and 1,400 mm in the northeast (Catarino *et al.*, 2001). The temperature shows little annual variation, with an average temperature of 26.5°C and a range between 25.9°C and 27.1°C.

The geomorphology of the territory exhibits a smooth relief. The coastal areas are mainly lowlands and the inner central and northeastern regions comprise plains that do not exceed 100 m in altitude. The Boé Hills, in the Southeast are the most elevated part of the territory, reaching 298 m. The hydrographic network, which is conditioned by the altitude of the territory and by marine transgression, is complex and extensive (Catarino *et al.*, 2008). Most of the fresh watercourses dry up by the end of the dry season. However, there are permanent fresh watercourses in the country: the Corubal, the Farim and the Geba rivers (Alves, 2000). The estuaries and inlets (locally called rivers) penetrate deeply in the continental territory. In addition, there are some small lakes in the country in the southern and eastern regions (Alves 2000).

Guinea-Bissau has been included in the Guinea-Congolia/Sudania regional phytogeographic transition zone (Catarino *et al.*, 2001). The country, despite its small size, shows great heterogeneity in flora and vegetation, which is related with climate and soils types (Catarino *et al.*, 2008). Two main phytogeographic areas can be distinguished: i) the

² <http://www.nationsencyclopedia.com>

coastal region of the continental territory and the Bijagós Archipelago, which are mostly occupied by forest-savannah woodland mosaics and ii) the east continental area, which vegetation is formed by savannah and savannah woodlands. In the south coastal area, the most northern extension of the Guinean forests occurs. This part of Guinea-Bissau retains important patches of dry and sub-humid forest patches (Catarino *et al.*, 2001). The vegetation present in the east continental region resemblances the Sudanese type since the climate is drier and the soils are shallow. Moreover, the coastline areas subject to the tide effect are occupied by great extent of mangroves and aquatic vegetation (Catarino *et al.*, 2001; Catarino *et al.*, 2008).

2.1.1 – Type of samples and sampling strategy

This study used three types of samples: faecal samples (from social units), hair samples (collected from captive individuals living as pets) and tissue samples (collected at bushmeat markets in the capital, Bissau).

Non-invasive faecal sampling in Guinea-Bissau focussed on social units, defined here as a group of baboons that forage/sleep together in the sampling day (e.g. faecal samples found in close proximity, no more than 10 meters apart). Sampling was planned in the south and east of the country where Guinea baboons are reported to exist (Gippoliti and Dell’Omo, 2003; Cá, 2008). Sampling focussed in three different sites: Cantanhez Woodlands National Park, Cufada Lagoon Natural Park and in the Boé Region (Fig. 2.1). Sampling locations are described in this section, along with a description of local biodiversity, the main habitat features and human density.

The areas visited by baboons were not specified at the beginning of this project. Initially, the local villagers and the guards and guides of the parks were interviewed and the areas they indicated were visited. Usually these locations corresponded to the baboon’s foraging areas (e.g. croplands, mangrove margins) or putative sleeping sites. These locations in Cantanhez Woodlands National Park were located very close to each other. Therefore, sampling of two social units that belonged to the same social group would in principle be possible or even likely. The home range described for the subspecies is of 25 Km² (Fickenschner, 2010), Guinea baboons are thought to travel 40 Km per day in savannah habitat (Galat-Luong personal communication) and their range size was estimated to be of 8 Km² (Dunbar, 1988). To circumvent this problem, a grid of 4x4 Km² was superimposed on the map of Cantanhez Woodlands National Park and only one social unit was sampled per square

(see Fig. 2.2). Also, whenever possible, samples of different squares were collected on the same day to assure the independence of units sampled. In Cufada Lagoons Natural Park and Boé region, the distance between groups was larger and it was not necessary to use the same strategy. The baboons in Cantanhez Woodlands National Park, in particular, had a propensity to run away very fast when approached. Therefore, a *wahoo* vocalization (similar to a dog bark) was imitated for a short period of time during which baboons would hopefully stand still and defecate.

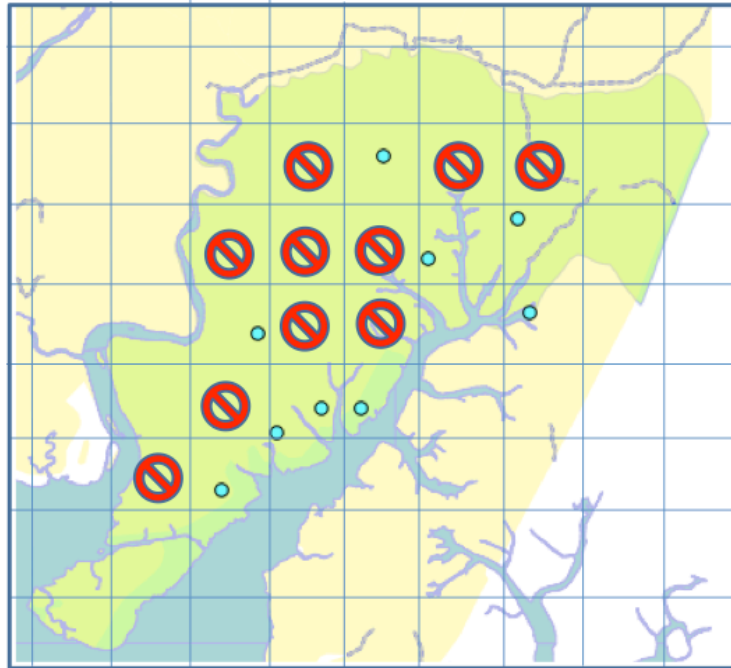


Figure 2.2: Sampling locations and effort for the Cantanhez National Park (⊘ indicate the quadrats where the baboons were absent and ● indicate quadrats where baboons were present).

The faecal sample collection occurred in two phases. At the beginning of the project (September to November 2008) faecal samples were collected in Cantanhez Woodlands National Park to perform a pilot study and to optimize laboratory procedures (see Appendix 2). The majority of samples analysed in this project were collected in the second sampling phase (January to June 2010). According to the results obtained in the pilot study (Appendix 2), samples collected in the second sampling phase were preserved using the “two-step protocol” (Roeder *et al.*, 2004). Nearly a teaspoon (5 ml) of faecal material was collected from the exterior part of the sample, by scraping the surface using a wooden stick, and immediately immersed in 99% (ANALAR) ethanol, where it remained for 24-48h. After that period, the samples were transferred to a tube containing 30g of Silica Gel (Type III, S-7625, indicating for desiccation, Sigma-Aldrich ® Company Ltd, Dorset, UK) and maintained at

room temperature until DNA extraction. During all procedures, gloves, facemasks and hairnets were used to limit possible contamination. During sample collection, the GPS location of each social unit was recorded along with observational notes: freshness of samples, size of group if observed and the relevance of the sampling area for baboons (sleeping site, foraging area, etc). I aimed to collect 30 samples per social unit.

Along with the faecal samples, eight hair samples from captive individuals were obtained. These samples were taken during grooming sessions from young individuals (babies or infants), living as pets in Bissau (4 samples) and Buba (2 samples). The sex, location and origin of the individuals were registered. The samples were kept in paper envelopes at room temperature until DNA extraction.

Additionally, 14 tissue samples were collected in a bushmeat market in Bissau during a bushmeat trafficking study (Chapter 3). The samples were preserved in 99% (ANALAR) ethanol at room temperature until DNA extraction.

The majority of faecal samples analysed in this project came from Guinea-Bissau. However, it was also possible to process few faecal samples from Mauritania as part of a collaboration with José Carlos Brito (researcher at CIBIO/U.Porto, *Portugal*). Samples were already desiccated by the dry air of the Mauritanian desert and so, were simply stored in plastic bags until DNA extraction.

2.1.2– Sampling sites and Effort

2.1.2.1 – Cantanhez Woodlands National Park

Cantanhez Woodlands National Park (*Parque Nacional das Matas de Cantanhez*: total area 106,767 Ha) is located in the south of Guinea-Bissau, in the administrative region of Tombali, comprising the Bedanda, Cacine and Quebo sectors. Within the park, there are about 20,000 residents in 13 villages. The main ethnic groups are the Balanta, the Nalus, the Tandas, the Djacancas, the Fulas and the Soussos. The Nalu people are considered the owners of the land (*donos di tchom*) because they are the oldest residents in the area³.

There are several ecosystems within this small area: sub-humid forest, mangrove forest (locally called “*tarrafe*”), wet grass savannah (locally called “*lala*”) and palm forest

³ <http://www.ibap-gbissau.org>

(dominated by *Elaeis guineensis*) (Fig. 2.3). These heterogeneous ecosystems attract a high diversity of flora and fauna, e.g. 30 mammal species have been inventoried (including the chimpanzee and guinea baboon, along with other primate species)².

During the fieldwork seasons of 2008 and 2010 (in a total of 150 days), I collected 86 and 357 samples, respectively. In total, 14 social units were sampled (one unit per quadrat) (see geographic location in Fig. 2.3 and sampling effort in Table 2.1). Most samples were collected a few minutes after observation of the group. It was possible to confirm the absence of baboons from some quadrats, based on both local villager information and on several visits to those areas (between 3 to 6 visits) during which no evidence of baboons was found (Fig. 2.2).

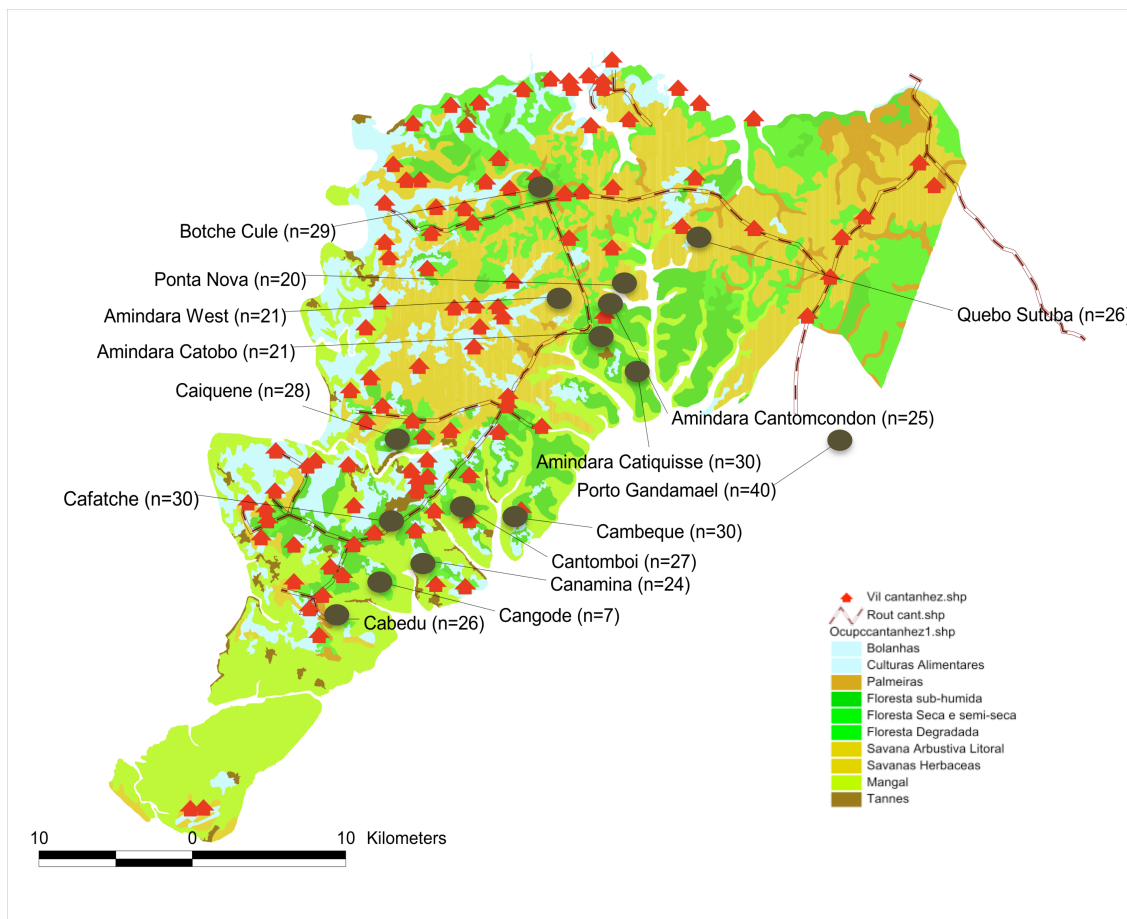


Figure 2.3: Location of sampled social units at Cantanhez Woodlands National Park and number of samples collected. Red symbols represent villages within the Park area (Map by INEP).

Table 2.1: Sampling locations, date, effort and sampling observations for Cantanhez Woodlands National Park

LOCATION	DATE	NUMBER	OBSERVATIONS
Cabedu	23-03	26	Foraging party after observation; Collected in the same day as Canamina group
Canamina	23-03	25	Foraging party, after observation; Collected in the same day as Cabedu group
Cafatché	27, 28 and 29-03	30	Foraging party, collected after observation in 3 following days
Cantomboi	8-02	26	Foraging party, samples with less than one day old
Caiquene	1-02	27	Foraging party, samples with less than one day old
Cambeque	15-02	30	Foraging party, samples with 12 hours old.
Amindara-Catiquisse	23-03	30	Collected in a drinking spot, after observation
Amindara-Catobo	28-01	21	Foraging party, after observation of the group
Amindara-Catomcondon	13-02	22	Foraging party, after observation
Amindara-West	11-04	21	Foraging party, sampled after observation
Botche-Culê	29-04	28	Foraging party, sampled after observation
Quebo-Sutuba	10-03 and 20-03	26	Foraging party (samples with one day old) and sleeping site, sampled after observation.
Quebo-Sutuba - Cancire	17-03	31	Foraging party, after observation
Gandamael Porto	18, 19, 20 and 21-05	39	Foraging party after observation; Collection of 39 samples took 4 days
Total		357	

2.1.2.2 – *Cufada Lagoons National Park*

The Cufada Lagoons Natural Park (PNLC – Parque Natural das Lagoas da Cufada: total area of 89,000 Ha) is located in the south, in the administrative region of Quinará, delimited by the Fulacunda village in the east, by the Buba village in the northeast and by the Corubal river in the north and by the big river of Buba in the south. The Park was created in December 1999. There are around 3,500 residents distributed in 33 villages mostly concentrated in the north, along the Corubal River, or along the road crossing the park, between Buba and Fulacunda villages (Fig. 2.3). Inside the Park area there are important wetlands: the “Bionra”, the “Bedasse” and the “Cufada” lagoons, considered Ramsar sites. The Cufada lagoon is the biggest lagoon in the country, supporting various species of birds along with hippopotamus, crocodiles and manatees. In addition, several species of primates (including the chimpanzee and the guinea baboon) are present⁴.

⁴ <http://www.ibap-gbissau.org/>

During 25 days I was able to collect 150 samples in Cufada (Fig. 2.3). From these, 120 samples were collected inside the Park area (four social units) and 30 samples were collected outside the Park area (one social unit) (Table 2.2). Most samples were collected after observation of groups, mainly formed by foraging parties. Nevertheless, at Bubatchingue site, sampling occurred at a sleeping site. To make the sampling comparable with the other locations, samples in Bubatchingue were collected in close proximity to each other, assuming that individuals that would forage together during the day would sleep close to each other at the sleeping site (Anderson and McGrew, 1984).

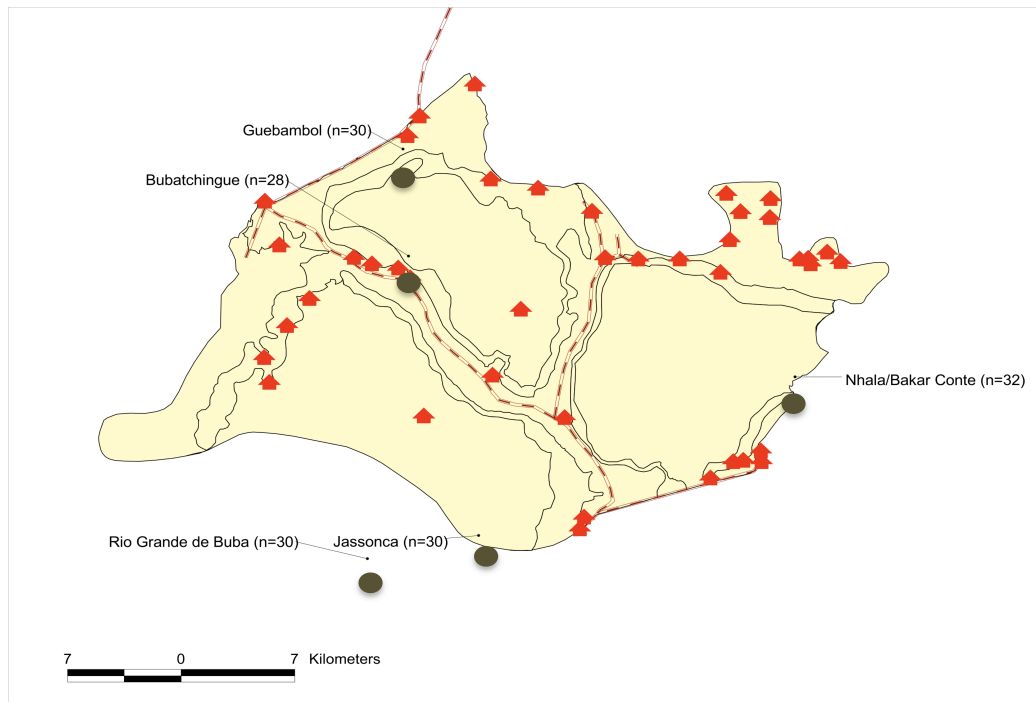


Figure 2.3: Sampling locations and effort for Cufada Lagoon Natural Park (Red symbols represent villages). Main roads within the Park are indicated) (Map by INEP).

Table 2.2: Sampling locations, date, effort and sampling observations for Cufada Lagoon Natural Park

LOCATION	DATE	NUMBER	OBSERVATIONS
Guebambol	26-04	30	Foraging party, samples with less than 12 hours
Bubatchingue	20-04	28	Sleeping site, after observation
Bakar Contê	22-04	32	Foraging party after observation of the group
Rio Grande de Buba (Sr. Soares 2)	27-04	30	Foraging party, after observation of the group
Jassonca (Sr. Soares 1)	29-04	30	Foraging party, after observation of the group
Total		150	

2.1.2.3 – *Boé Region*

The Boé region is located in the southeast of Guinea-Bissau, in the Gabú Administrative region. This area is partially isolated from the rest of the country. In the west is delimited by the Corubal River and in the south and east by the border with Republic of Guinea. This area is in the process of becoming protected and the description of the present biodiversity is weak. Nevertheless, there has been confirmation for the presence of chimpanzees and baboons (Gippoliti and Dell’Omo, 2003) along with elephants, lions and leopards (Brugiere *et al.*, 2005; Brugiere *et al.*, 2006). The most common type of vegetation in this region is savannah woodland (Catarino *et al.*, 2001).

In total, 50 samples were collected from three social units (Fig. 2.4; Table 2.3), minutes after observation of groups.

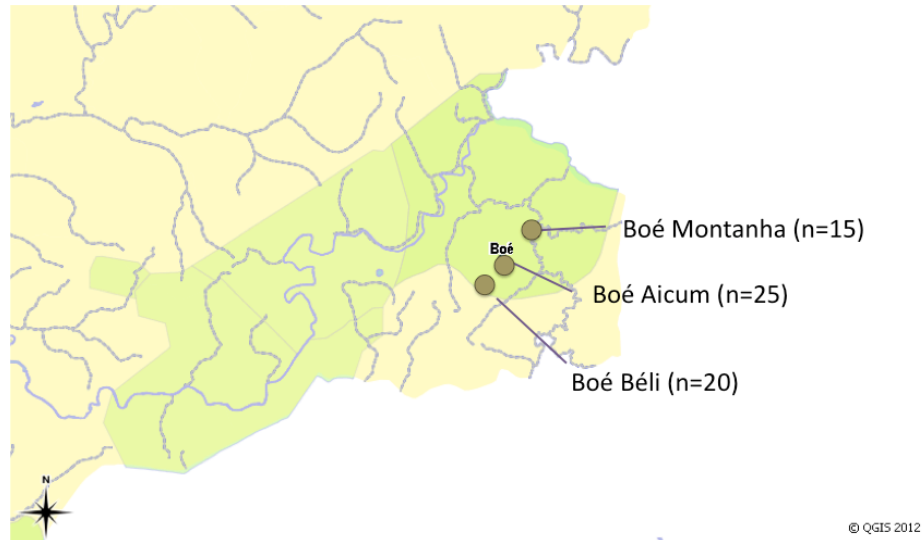


Figure 2.4: Sampling locations and effort for Boé Region

Table 2.3: Sampling locations, date, effort and sampling observations for Boé Region

LOCATION	DATE	NUMBER	OBSERVATIONS
Boé Beli	10 th May	20	Foraging party, after observation of group
Boé Aicum	11 th May	15	Foraging party, after observation of group
Boé Montanha	15 th May	15	Foraging party, after observation of group
Total		50	

2.2 – Laboratory methods

During the first period of the project, I conducted a pilot study to test several faecal sample preservation methods, two DNA extraction methods and to design PCR multiplexes and proceed with their optimisation (Appendix 2). This section describes the final laboratory procedures used.

2.2.1 - DNA extraction

2.2.1.1 – Faecal DNA extraction

Total genomic DNA was extracted using the QIAamp ® DNA Stool Mini Kit (QIAGEN ®) with some modifications from the manufacturer's protocol to maximize the amount of DNA extracted: i) only the outer part of the faecal sample was used, having been scraped with a blade; ii) the sample was immersed in ASL Buffer overnight (instead of just one minute); iii) after overnight immersion in ASL Buffer, an additional quantity of ASL Buffer was added to the sample if all the liquid was absorbed (usually between 200ul and 600ul or up to the 1.4 ml of liquid necessary for the next step); iv) the period of action for the InhibitEX Tablet was increased for a total of 10 minutes (instead of just 1 minute); v) the incubation period with Proteinase K was increased to 30 minutes (instead of just 10 minutes). DNA extracts were eluted in 200ul Buffer AE, as recommended by manufacture's manual. After aliquotating the samples, the stock was stored at -20°C.

Several precautions were taken to avoid human contamination during DNA extraction procedure. Extractions were conducted in a laminar-flow hood. Before extraction, all material was subjected to the UV irradiation for at least 30 minutes and the inside of hood was cleaned with bleach (10% dilution). Head and facial masks were used at all times and nitrile gloves were frequently changed, in particular between the “scraping” phase of faecal samples and whenever contamination of gloves with liquid occurred. The forceps and blades were washed in 100% bleach, rinsed with alcohol and blue-flamed between samples. Only sterile filter tips were used during all steps of extraction.

One negative control per 12 samples was subjected to all the extraction procedures (and was included in the PCRs) to test for the possible contamination with human DNA and/or cross-contamination between samples. In total, 480 faecal samples were extracted: 464 faecal samples collected in Guinea-Bissau and 16 faecal samples collected in Mauritania (see

table 2.4 for details on the number of samples per social unit). All the samples collected in Cufada Lagoons Natural Park and in Boé region were extracted. In Cantanhez Woodlands National Park, a sample of the best preserved samples and the most distinct social units were extracted.

Table 2.4: Details on number of faecal samples extracted of each social group for Guinea-Bissau and number of DNA samples obtained from hair and tissue samples.

Country	Site	Location	Type of samples	N
Guinea-Bissau	Cantanhez	Amindara Catobo	Faecal	21
		Caiquene		27
		Cantomboi		26
		Amindara Catomcondon		4
		Cambeque		30
		Cabedu		26
		Canamina		25
		Quebo Sutuba		17
		Botche Cule		28
		Porto Gandamael		40
		Ponta Nova		20
	Cufada	Bubatchingue		28
		Nhala/ Bakar Contê		32
		Guebombol		30
		Sr. Soares 1		30
		Sr. Soares 2		30
		Boé_Beli		20
	Boé	Boé Aicum		15
		Boé Montanha		15
	Buba	Captive individuals	Hair	2
	Bissau	Captive individuals		4
		Bushmeat markets	Tissue	14

2.2.1.2 – Hair DNA extraction

Hair samples were extracted according with the protocol designed by Fairus Jalil, PhD (Fairus's Lab survival manual), which was based in Allen *et al.* (1998) and Vigilant (1999). All material used (e.g. forceps and blades) were washed in 100% bleach, rinsed with alcohol and blue-flamed between samples. Extractions were conducted in a laminar-flow hood, previously washed with 10% bleach. The hairs' root was cut into an eppendorf containing 20ul PCR Buffer (10x), 1ul Proteinase K (50ug/ul) and 79ul water. The mixture was agitated overnight at 37°C and then maintained at 100°C for 10 min.

2.2.1.3 – Tissue DNA extraction

Whole genomic DNA was extracted using the DNeasy Blood and Tissue kits (Qiagen©), following the manufacturer's protocol with one slight modification (overnight lysis of tissues instead of just 30 minutes). DNA extracts were eluted in 200ul Buffer AE and stored at -20°C. The samples were subjected to molecular identification, using a mitochondrial DNA COI gene fragment and 12S rRNA gene fragment (Chapter 3). An undergraduate student, Emily Wallace, extracted the DNA and performed the molecular identification of bushmeat samples during her final year project (Wallace, 2011).

2.2.2 – DNA amplification

2.2.2.1 – Microsatellite loci

Fifteen autosomal microsatellite markers, amplified in 5 multiplex and one singleplex PCR, were used to genotype the samples. Most microsatellite loci were tetranucleotide repeats, with the exception of D7S503, which is a dinucleotide repeat locus. Allele range size varied between 125 base pairs and 250 base pairs (bp) (see Table 2.5 for details of each multiplex). Microsatellites were human-derived with cross-amplification in genus *Papio* (e.g. Bayes *et al.*, 2000).

The microsatellite loci were amplified in 10uL volume using the QIAGEN Multiplex PCR Kit ®, containing 2uL of DNA extract. Following the manufacturer's instructions, the final reaction concentrations consisted of 1x QIAGEN Multiplex PCR Master Mix ® and 0.2uM of each multiplex primer mixture. 0.75uM of BSA was also added. In the multiplexes, different concentration of each primer was used, accounting for differences in amplification success (Table 2.5).

All multiplex PCR cycling conditions started with a HotStarTaq DNA Polymerase activation step of 15 min at 95°C, followed by 40 cycles of denaturation step at 94°C for 30 sec, annealing step for 40 sec at between 50°C to 59°C (depending on the multiplex) and extension at 72°C for 60 sec. The PCR ended with a final extension of 30 min at 72°C. PCRs were performed in an AB Applied Biosystems™ (California, USA) Veriti 96 Well Thermal Cycler.

In order to avoid cross-contamination between samples and possible external DNA contaminations, all material used in PCRs was subjected to UV decontamination for 15

minutes, PCRs were always assembled in a Microflow OMNI PCR workstation (Bioquell UK Ltd, Hampshire, UK), sterile filter tips were used in all steps of PCRs, gloves were changed before the start of the PCR and both extraction and PCR negatives were included in the reactions.

Amplification was tested using 2uL PCR product subjected to a 2% agarose gel electrophoresis (120V) and then visualized with 0.1 mg/ml ethidium bromide on a 3 UV transilluminator (UVP Gel doc it TM, Cambridge, UK). All samples were analysed using MacroGen's Genescan service and run on an ABI3730XL capillary analyser. Multiplex 1, 3, 4 and 5 were analysed using a 16 GeneScanTM -500 LIZ ® size-standard and multiplex 2 was analysed using 16 GeneScanTM -400 HD ® size-standard.

2.2.2.2 – Mitochondrial DNA

A fragment of approximately 490 bp of the mitochondrial DNA (mtDNA) control region (hypervariable region I) was amplified and sequenced using the external primers designed and published by Hapke *et al.* (2001): L15437: CTGGCGTTCTAACTTAACT and H15849: GTAGTATTACCCGAGCGG. By using these pair of primers, the results obtained by this study can be directly compared with those obtained by Hapke *et al.* (2001) and Burrell (2008) and with Guinea baboon sequences obtained by Dietmar Zinner (who facilitated sequences from Senegal and Republic of Guinea as part of a collaboration established with the German Primate Center).

The mtDNA fragment was amplified in a 22uL volume using the QIAGEN Multiplex PCR Kit ®, containing 1.5uL of DNA extract. Following the manufacturing instructions, the final reaction concentrations consisted of 1x QIAGEN Multiplex PCR Master Mix ® and 0.2uM of each primer. 0.75uM BSA was also added. The PCR started with a HotStarTaq DNA Polymerase activation step for 15 min at 95°C, followed by 40 cycles of denaturation step at 94°C for 30 sec, annealing temperature at 59°C for 90 sec and extension at 72°C for 60 sec. The PCR ended with a final extension of 30 min at 60°C. PCRs were performed in an AB Applied BiosystemsTM (California, USA) Veriti 96 Well Thermal Cycler. In order to avoid cross-contamination between samples and possible external DNA contaminations, the procedures referred in section 2.2.2.1 were implemented.

Chapter 2: General Methodology

Table 2.5: Details of Multiplexes PCRs (annealing temperature): loci included in each multiplex, name and GenBank code, primers sequences, repeat motif, colour of fluorescent dye, final PCR concentration, allele range for Guinea-Bissau population and Size Standard. Note that singleplex PCR has the same annealing temperature as M5

MULTIPLEX	LOCUS	GENBANK CODE	PRIMER F PRIMER R (5'-3')	REPEAT MOTIF	DYE	FINAL CONC (uM)	RANGE (bp)	SIZE STAND.
M1 57°C	D13S765	G09003	TGTAACCTTACTTCAAATGGCTCA TTGAAACTTACAGACAGCTTGC	GATA	NED and TAMRA	0.15	200-212	LIZ_400
	D12S375	G08036	TTGTTGAGGGTCTTTCTCCA TCTTCTTATTTGGAAAAGTAACCC	GATA	PET	0.1	164-184	
	D3S1766	G08269	ACCACATGAGCCAATTCTGT ACCCAATTATGGTGTGTTACC	ATCT	FAM	0.1	192-208	
	D7S503	G18277	ATGACTTGGAGTAATGGG AACCTTTAATCAGGATACAGAC	CA	NED	0.6	142-156	
M2 55°C	D2S1326	G08136	AGACAGTCAAGAATAACTGCC CTGTGGCTCAAAAGCTGAAT	CTAT	FAM	0.3	192-208	HD_400
	D14S306	G09055	AAAGTACATCCAAATAGGTAGG TGACAAAGAACTAAAATGTCCC	GATA	FAM	0.2	161-181	
	D1S533	G07788	CATCCCCCCCCAAAAATATA TTGCTAATCAAATAACAATGGG	GATA	HEX	0.4	187-203	
M3 59°C	D8S1106	G09378	TTGTTTACCCCTGCATCACT TTCTCAGAATTGCTCATAGTGC	GATA	VIC	0.1	149-161	LIZ_400
	D6S501	G08551	GCTGGAACTGATAAGGGCT GCCACCCTGGCTAAGTTACT	CTAT	FAM	0.5	171-187	
	D10S611	G08794	CATACAGGAACTGTGTAGTGC CTGTATTTATGTGTGTGGATGG	GATA	FAM	0.1	129-137	
M4 57°C	D5S1457	G08431	TAGGTTCTGGGCATGTCTGT TGCTTGGCACACTTCAGG	GATA	PET	0.1	125-137	
	D7S2204	G08635	TCATGACAAAACAGAAATTAAGTG AGTAAATGGAATTGCTTGTACC	AGAT	FAM	0.4	230-250	
	D3S1768	G08287	GGTTGCTGCCAAAGATTAGA CACTGTGATTTGCTGTTGGA	GATA	VIC	0.1	193-212	
M5 58°C	D21S1442	G08071	CTCCTCCCCACTGCAGAC TCTCCAGAATCACATGAGCC	GATA	FAM	0.4	221-245	LIZ_400
	Sex_DET	C. Roos	GGACGRACTCTAGATCGGTA GTNCAGATCTARGAGGAAGC	No repeat motif	PET	0.2	150 or 180	
SINGLEPEX	D4S243	M87736	AATCCCTTTTCTACCTTTCTATCAC GAGAGGAGAGATAAAAGATGTAAATG	GATA	FAM	0.2	152-172	HD_500

Amplification was tested using 2uL PCR product subjected to a 2% agarose gels electrophoresis (120V) and then visualized with ethidium bromide in a 3 UV transilluminator (UVP Gel doc itTM, Cambridge, UK). PCR products were purified with 10 units of Exonuclease I and five units of Antarctic Phosphatase (New England Biolabs Inc., Ipswich, UK). Cycling conditions for the PCR products purification started with a 30 minutes step at 37°, followed by 20 mins at 80° and 5 mins at 12°C. Samples were then sequenced bidirectionally by Macrogen © Europe's EZ-seq direct service, using an ABI3730XL capillary analyzer.

2.2.2.3 – Molecular Sex Determination

The sex of the individuals was identified using a molecular protocol designed by Christian Roos (unpublished). This protocol uses two primers designed on the Dead Box gene (DDX3X) (F: GGACGRACCTAGATCGGTA, R: GTNCAGATCTARGAGGAAGC). The primers amplify two fragments in the male (with a length of 150 bp and 180 bp) and only one fragment in the female (with a length of 180 bp). Although this small difference (30 base pairs) can be visualized on agarose gels (with a run time of 30 minutes at 120 volts), the forward primer was end-labelled with a PETTM fluorescent dye (Applied Biosystems) and the sex-determination marker was included in Multiplex 5. This procedure avoided possible non-detection of the male diagnostic band in agarose gels since fragment analysis have a much higher resolution.

2.2.3 – Genetic quality control

2.2.3.1 – Microsatellite loci genotyping: minimising errors during data production

This section describes the methodological strategies employed to minimise genotyping errors.

Genotyping errors (an individual genotype that does not correspond to the true one (Bonin *et al.*, 2004) can be grouped in four categories: i) related to the underlying DNA sequence (e.g. null alleles - the non-amplification of alleles due to mutations occurred at the flanking region of the genetic markers) and size homoplasy (the scoring of two different alleles that have the same fragment size), ii) related to the low quantity/quality of the DNA extract (allelic errors, including large allele dropout and the *in vitro* generation of false alleles), iii) biochemical artefacts and iv) human errors (Pompanon *et al.*, 2005). The results

obtained by studies in which individual identification, population structure and assignment and kinship are aimed, as in this project, can be greatly biased by errors in genotyping (Boninet *et al.*, 2004; Pompanon *et al.*, 2005). Complete error elimination is not possible because the procedures are not 100% trustworthy (Bonin *et al.*, 2004).

Therefore, specific measures were designed from the early stages of the project to: i) limit errors during the production of genotypic data, ii) identify the samples with putative errors and remove them from the dataset (Pompanon *et al.*, 2005). Human errors and low DNA quantity/quality related errors are the most common source of errors (Bonin *et al.*, 2004). The following sections describe the procedures used to limit them and the procedures to remove the genotyping errors from the dataset.

2.2.3.1.1 – Limitation of genotypic errors during data production

2.2.3.1.1.1 – Human error

To limit this type of error the following strategy was used:

- a. The different procedures (DNA extraction, DNA amplification and pos-PCR procedures) were performed in different rooms (Bonin *et al.*, 2004).
- b. Samples were processed in batches, following the order of DNA extraction to limit possible sample swaps or misidentification;
- c. All amplification repeats were done at the same time for a certain multiplex in order to limit number of times a sample is handled;
- d. Bins were created for each allele to eliminate the inclusion of non-baboon alleles or a lack of consistency in the conversion of raw decimal data into integers (DeWoody *et al.*, 2006);
- e. Allele scoring followed a semi-automated procedure (automated allele calling followed by visual examination) to reduce errors related with non-detection of new alleles, amplification of PCR artefacts or possible errors related with stutter patterns (DeWoody *et al.*, 2006);
- f. Whenever necessary, allele calling was checked by an independent observer.

To create bins with the software GeneMapper® ID version 3.2 (Applied Biosystems), the true allele size calling (size of the fragment amplified with decimal values) was registered for the first 80 samples that were used as reference genotypes (following

recommendations described by Guichoux *et al.*, 2011). The allelic binning (the conversion of the values of fragment size in discrete units, Idury and Cardon, 1997) was accomplished by calculating the average value for each allele, allowing a variance of 0.4.

To prevent the misidentification of a human allele with a baboon allele (as the set of microsatellite loci used were first designed for humans), a human DNA extract of the main researcher (and the only one manipulating the samples at the lab) was genotyped. The bins for the human alleles were also created in the software GeneMapper® ID version 3.2 (Applied Biosystems). Although it could be argued that the alleles scoring using bins might result in the non-identification of some microvariant alleles (resulting from a mutation in the flanking region, Guichoux *et al.* 2011), the contamination by human DNA was of a much higher concern and was thus prevented as far as possible.

2.2.3.1.1.2 – Low DNA quantity or sample quality-related errors

To limit genotyping errors due to low DNA quantity or quality, obtained from faecal sample extractions, the following strategy was used:

- a. The genotyping procedure followed the “multi-tubes” approach (Taberlet *et al.*, 1996) in order to define the consensus genotype of each sample per locus. In this approach, the sample is amplified multiple times for each locus and the comparison of the different replicates reveal the true genotype (Dewoody *et al.*, 2006). Since the cost and effort associated with the protocol of Taberlet *et al.* (1996) was prohibitive in the present study (amplification of homozygotes individuals up to seven times), the number of replicates was estimated taking into consideration the allelic dropout and false alleles error rates in the data, using a maximum likelihood approach (Pompanon *et al.*, 2005).
- b. Low quality genotypes were identified and removed. Each sample was classified according with the reliability of its genotype, using a quality index across loci calculated according to Miquel *et al.* (2006). Samples considered to represent the most unreliable genotypes were eliminated from the dataset.

This strategy was divided in two phases in order to distinguish low-quality samples as early as possible in the process and remove them from subsequent analyses (Paetkau, 2003). First, all samples were amplified using Multiplexes 1 and 4 for four replicates (see below for more information on the number of repeats). The loci included in those

Multiplexes proved to have a higher amplification rate and could therefore provide sensitive information about DNA quality. All samples with a quality index above 0.50 across the nine loci (in Multiplexes 1 and 4) were further used to amplify the other Multiplexes. All other samples with a lower quality index were removed. Second, at the end of the molecular analysis, the total quality index (for all 15 loci) was recalculated and samples with a quality index below 0.55 were removed from the dataset (as recommended by Miquel *et al.* 2006).

The number of replicates necessary to obtain 95% confidence in the genotypes was estimated using the allelic dropout and false allele rate from the pilot study samples. These error rates were estimated locus-by-locus using a maximum likelihood approach (Johnson and Haydon, 2007a) implemented in software Pedant version 1.0 (Johnson and Haydon, 2007b). With this approach, only two amplifications per loci were required to estimate the frequency and type of mismatches between the duplicates along with the respective confidence regions (Johnson and Haydon, 2007a).

The maximum likelihood method underestimates error rates in sets of low quality samples, which will have both allelic dropout in both amplifications per locus and where false alleles will be undetectable (because of the high rate of allelic dropout). Therefore, it is recommended the use of at least 25 samples from the same population, the elimination of low quality samples in the estimation of the error rates and the use of samples with similar quality (Johnson and Haydon (2007a; Johnson and Haydon 2007b). For that reason, duplicate amplifications for fifteen microsatellite loci for 80 samples from Cantanhez Woodland National Park (assumed to belong to the same population) with a quality index (Miquel *et al.*, 2006) above 0.50 were used. The error rates estimated by Pedant software are shown in Table 2.6.

The number of necessary replicates was then estimated using GEMINI version 1.4.1 (Valière, 2002, <http://nath.valiere.free.fr/Gemini>). First, the minimum number of replicates that confirms an allele per locus (“Consensus Threshold”) based in the allelic dropout rate and false allele rate was estimated for values between two and 12, using 100 simulations. Using the “PCR repetition module”, a plot of the accuracy of the results with the increasing number of PCRs replicates was obtained. An asymptote was reached at four replicates across loci suggesting that increasing the number of replicates would not significantly increase the reliability of genotypes. Therefore four replicates across loci would assure a

95% confidence on the genotypes (with a remaining 5% error remaining). Consequently, four amplifications per locus per sample were carried out.

The consensus threshold generated for the four replicates was also used as a set of rules to genotype the samples (see Table 2.6): i) an allele was confirmed if appearing at least the number of times indicated by the consensus in four amplifications (which could vary across the loci, reflecting the respective error rates); ii) homozygote individuals confirmed by the minimum of three amplifications and one non amplification were accepted if the consensus threshold was of two; iii) special attention was given to loci with a consensus threshold of one; these loci were only genotyped in the case of four positive amplifications; in the extreme situation of three amplifications with one allele and the fourth amplification with a second allele, the PCR was repeated to assure that the allele observed only once was not a result of a contamination. The result of the sex determination protocol was considered as a true if observed at least three times over the four repeats performed.

Finally, the quality of genotypes included in the dataset was estimated using a “quality index” (Miquel *et al.*, 2006). According with this method, the amplifications performed per locus receive a score: one if it equals the consensus genotype or zero if it is different (zero is assigned to all possible cases, including non-amplification, allelic dropout or a putative false allele; Miquel *et al.*, 2006). The quality index per locus was obtained by dividing the sum of scores by the number of amplifications performed and used to reach consensus genotype. Furthermore, the average of quality index across loci was obtained to evaluate the reliability of genotypes per sample (Miquel *et al.*, 2006). Only samples with a quality index above 0.55 were selected to be included in the final dataset as recommended by the authors (Miquel *et al.*, 2006).

2.2.3.1.2 – Eliminating the database errors

A two-level approach was carried out to detect errors. The first level was focussed in identifying allele-scoring related errors. In the second level, the database was tested for widespread genotyping errors (in particular allelic dropout and null alleles).

All non-concordant replicates of the database were checked, which involved re-evaluation of the allele calling. This also decreased the number of possible mismatches in the data entry (Bonin *et al.*, 2004). After, the chromatograms of all samples with private alleles (identified using software GenALEx v. 6.3, Peakall and Smouse, 2006), and

genotypically distinct samples (identified using a factorial correspondence analysis, done using the software Genetix 4.05 (Belkhir *et al.*, 1996-2004), were re-evaluated.

Table 2.6: Allelic dropout and false alleles rates estimated per locus by Pedant version 1.0 and the respective Consensus Threshold calculated by GEMINI version 1.4.1.

Locus	Allelic dropout	False allele	Consensus
D12S375	14	2	2
D7S503	18	0	2
D3S1766	13	1	2
D13S765	28	0	1
D10S611	15	0	1
D6S501	22	0	1
D8S1106	9	0	1
D3S1768	17	0	2
D7S2204	16	0	1
D5S1457	24	2	2
D21S1442	16	2	2
D4S243	11	4	2
D2S1326	28	0	1
D14S306	18	1	2
D1S533	31	4	2
Average across	19	1	

In the second level, the genotyping results were tested using two statistical tools: Excel Microsatellite toolkit (Park, 2001) and Micro-checker (van Oosterhout *et al.*, 2006). The analysis performed in Excel microsatellite toolkit (Park, 2001) allowed the identification of typing errors and identical genotypes (allowing the elimination of repeated sampled individuals). Using Micro-checker v2.2.3 (Van Oosterhout *et al.*, 2006) allowed the detection of putative null alleles, scoring errors due to stuttering and large-allele dropout. Analysis was done using the whole dataset (N = 163) and a 95% confidence interval. This software, in particular, tests the raw genotypic data for conformity with Hardy-Weinberg equilibrium since a deficiency in heterozygotes can indicate the presence of errors (Dewoody *et al.*, 2006; Selkoe and Toonen, 2006) in particular allelic dropout and null alleles (Paetkau, 2003).

It can be argued that, instead of genotyping errors, demographic or mating system processes can lead to an excess of homozygosity. For example a Wahlund effect (when two or more populations are analysed as a single group) or a high level of inbreeding can lead to homozygosity excess. These factors will induce a lack of conformity to what is expected

under a Hardy-Weinberg population (random mating, no migration, no drift and no mutation) (Dewoody *et al.*, 2006; Selkoe and Toonen, 2006). However, such demographically-mediated deviations should be reflected across most or all loci analysed and not in a subset of loci (Dewoody *et al.*, 2006; Selkoe and Toonen, 2006). In addition, the presence of null alleles at one or a few loci can also lead an excess of homozygotes. It is expected that heterozygotes samples for null alleles will be homozygotes in the database and homozygotes samples for null alleles will not show any product in the amplification (Dewoody *et al.*, 2006). The presence of null alleles in the database will also bias the alleles frequencies and thus decrease the number of heterozygotes for a few loci (Dewoody *et al.*, 2006).

The allelic dropout (ADO) and false allele rate (FA) of the overall data was estimated using equations 2 and 3 in Broquet and Petit (2004). ADO was estimated per locus, as the number of replicates with one missing allele of the consensus genotype (e.g. replicates where the allelic dropout was visible) as a proportion of all positive amplifications of heterozygotes (equation 2, Broquet and Petit, 2004). FA rate was estimated per locus as the number of replicates showing false alleles (e.g. any amplification with an allele not considered real) as a proportion of the total number of positive amplifications (equation 3, Broquet and Petit, 2004).

2.2.3.2 – Mitochondrial DNA post-sequencing procedures

Sequences were manually checked for accuracy using Sequencher version 4.9 (Gene Codes Corporation, USA). An alignment between forward and reverse sequences was made and a consensus sequence was created. All consensus sequences were then aligned and all polymorphic positions, including substitutions and/or indels were re-checked manually for each chromatogram. Sequences were then exported to BioEdit version 7.0.9 (Hall, 1999) and trimmed to 393 bp, corresponding to the length of the shortest sequence.

2.2.4 – Identification of individual profiles

Analysis of detection of repeated individuals, using the microsatellite data, was performed in Excel microsatellite toolkit (Park, 2001) allowing for one mismatch. In the case of individuals only distinguished by one homozygote locus, two mismatches were used. The analysis was then repeated in software GIMLET 1.3.3 (Valière, 2002). Duplicate genotypes were removed from the dataset.

The probability that two individuals sampled randomly from the population have the same genotype at all typed loci – the Probability of Identity (PI; Waits *et al.*, 2001) was estimated per locus. The loci were ranked according with their respective PI. This procedure was also done using a more conservative measure for populations with related individuals (PI between siblings). The software GIMLET 1.3.3 (Valière, 2002) was used to estimate both PI and PI_{sibs} .

2.3 – Results and Discussion

2.3.1 – Mitochondrial DNA

170 mitochondrial DNA sequences of 393 bp in length were obtained from 175 DNA faecal samples (corresponding to different individuals, see section 2.3.2.1). Additionally, 10 sequences obtained from six hair samples and four tissue samples were added to the final database. The DNA sequence analysed contained 56 polymorphic sites (with 58 mutations), 3 gaps and 2 sites with missing data. Thirty-nine haplotypes were found, with an average number of pairwise differences of 5.4. Across all samples, haplotype diversity (\pm SD) was of 0.82 ± 0.024 and nucleotide diversity was of 0.014 ± 0.00120 .

2.3.2 – Microsatellite loci

2.3.2.1 – DNA extraction and amplification success

In this study, 464 faecal samples collected in Guinea-Bissau were extracted and nuclear DNA was first amplified using multiplex 1 and 4. With the process that selected the most consistent samples, 258 samples (55.6% of samples extracted) were excluded due to non-amplification, low genotype consistency (quality index across loci below 0.50) or species misidentification. Further 45 samples were identified as repeated individuals and excluded from the dataset. In the end of the genotyping process only 35% of the samples extracted could be included in the dataset (161 samples of different individuals and quality index varying between 0.50 and 1) (Fig. 2.5).

After the estimation of error rates of this initial dataset, 12 samples with quality index ranging between 0.50 - 0.55 were further excluded, decreasing the allelic dropout rate (ADO) across loci from 18.0% to 15.6% and false alleles rate (FA) from 3.5% to 2.9%. Finally, a dataset with 149 different individuals samples was assembled with a quality index

between 0.55 and 1 (mean 0.82) (See Table 2.7 for samples in the final dataset, distributed in different social units).

All tissue samples (n=14) were successfully extracted and amplified and typed for loci. The hair samples, however, did not produce reliable genotypes and were excluded from the microsatellite dataset.

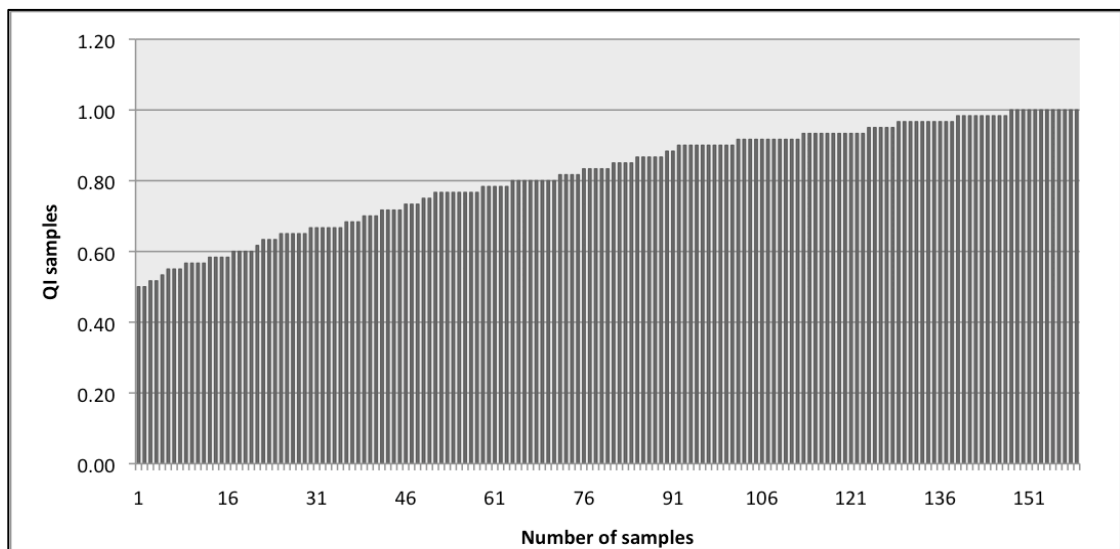


Figure 2.5: Quality index of 161 samples of different individuals (previous to exclusion of 12 samples with QI below 0.55)

Table 2.7: Final dataset: social units within sampling regions and respective sample size

Sampling regions	Name of social Unit	Number of samples
Cantanhez	Porto Gandamael	13
	Amindara Catobo	7
	Cabedu	9
	Catomboi	10
	Canamina	10
	Caiquene	4
	Cambeque	6
	Quebo Sutuba	4
	Botche Culê	10
Cufada	Bubatchingue	21
	Bakar Conte	11
	Guebombol	5
	Sr. Soares 1	7
	Sr. Soares 2	10
Boé	Boé Béli	6
	Boé Aicum	11
	Boé Montanha	5
Total		149

Mean amplification success across loci (estimated based in the proportion of positive PCR in all amplifications attempted) was of 81.2%. The amplification success varied greatly among loci, with locus D1S533 and locus D21S1442 showing the lowest success in amplification (only 58.9% and 63.9% of positive PCR respectively) and loci D4S243, D8S1106 and D6S501 showing the highest percentage of successful amplifications (94.4%, 94.2% and 93.8% of positive PCR respectively) (see Fig. 2.6). As found by other studies (Buchan *et al.*, 2005; Broquet *et al.*, 2007), loci amplifying smaller fragments generally showed a higher amplification success rate. However in this study, the correlation was weak ($R^2 = 0.13$) (Fig. 2.7).

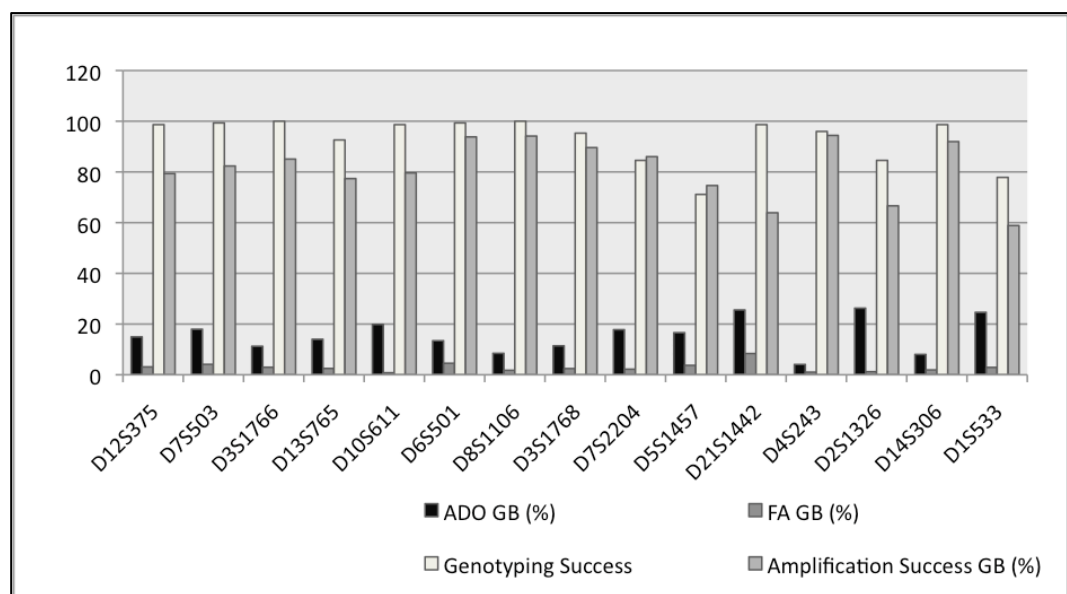


Figure 2.6: Per locus amplification success (%), genotyping success (%), allelic dropout (ADO) (%) and false alleles (FA) (%) rate

Amplification success for Guinea-Bissau faecal samples (81.2%) was in the range of what was found by other studies using baboon faecal samples. For example, Buchan *et al.* (2005) found 89% amplification success across 14 microsatellite loci with products sizes between 110 and 273 bp and Bayes *et al.* (2000) found 70% amplification success across eight microsatellite loci, with PCR products sizes between 122 and 192 bp. Nevertheless, this study found a lower amplification success than Fickenscher (2010) across the same 14 microsatellites loci and using the same amplification protocols (97.2% average amplification success across loci (Fig. 2.8).

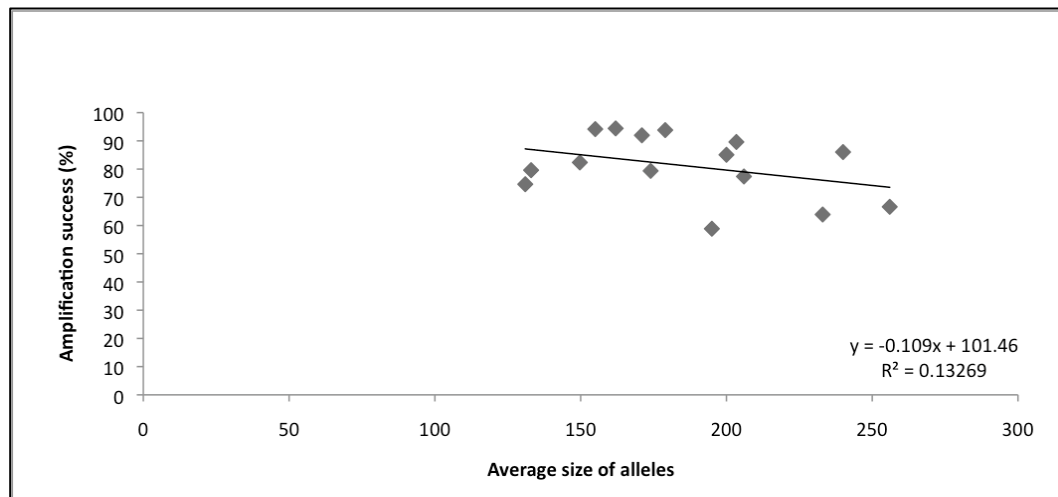


Figure 2.7: Correlation between amplification success (%) and the average size of alleles (bp)

Reasons that could account for such differences are likely to be related with lower quantity or quality of DNA and/or a higher concentration of PCR inhibitors in Guinea-Bissau baboon samples. It has been suggested that faeces can contain PCR inhibitors derived from the animal's diet that are not eliminated during the DNA extraction (Beja-Pereira *et al.*, 2009) although Brosquet *et al.* (2007) did not find any effect of diet on the nuclear DNA amplification success.

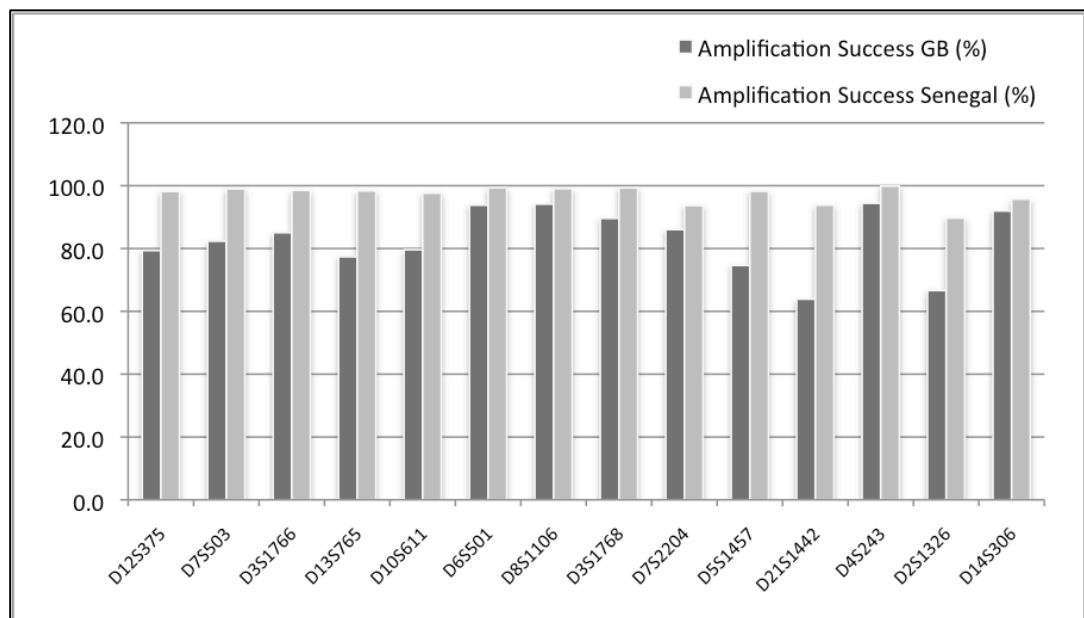


Figure 2.8: Amplification success (%) was lower in Guinea-Bissau samples than Senegalese samples (Fickenscher, 2010) across loci

Since both studies collected relatively fresh faecal samples and used similar desiccation protocols, the most likely explanation is the regional difference in the diet between Senegal and Guinea-Bissau baboons. Variation in diet could influence the quantity of PCR inhibitors and thus decrease the amplification success in this study. Furthermore, if this explanation has some empirical support, differences in the amplification success between sampling locations within Guinea-Bissau should be present, accounting for differences between sites in habitat and therefore in diet. In fact, samples collected in Cantanhez showed only 77% of amplification success and Cufada and Boé samples had a relatively higher success in amplification (82% and 80% respectively).

2.3.2.2 – Quality index, Null alleles, Allelic dropout and False alleles rates

The average quality index estimated across loci was 83%. Locus D21S1442 and locus D1S533 showed the least reliable genotypes ($QI_{D21S1442} = 53.4\%$ and $QI_{D1S533} = 63.1\%$) and D3S1766 and D14S306 possessed the most reliable genotypes ($QI_{D3S1766} = 94.5\%$ and $QI_{D14S306} = 94.1\%$) followed by locus D8S1106 and locus D3S1768 ($QI_{D8S1106} = 93.5\%$ and $QI_{D3S1768} = 93.3\%$). Amplification success and fragment size length did not have a strong linear relationship with the reliability of genotypes ($R^2 = 0.16$ and $R^2 = 0.68$, respectively). The quality index across loci decreased slightly with average size of alleles being amplified and with the amplification success (see Fig. 2.9).

The test carried out using Micro-checker did not reveal scoring errors due to stuttering or any evidence for large allele dropout. However, an excess of homozygotes in four loci (D10S611, D21S1442, D14S306 and D1S533) suggested the presence of null alleles.

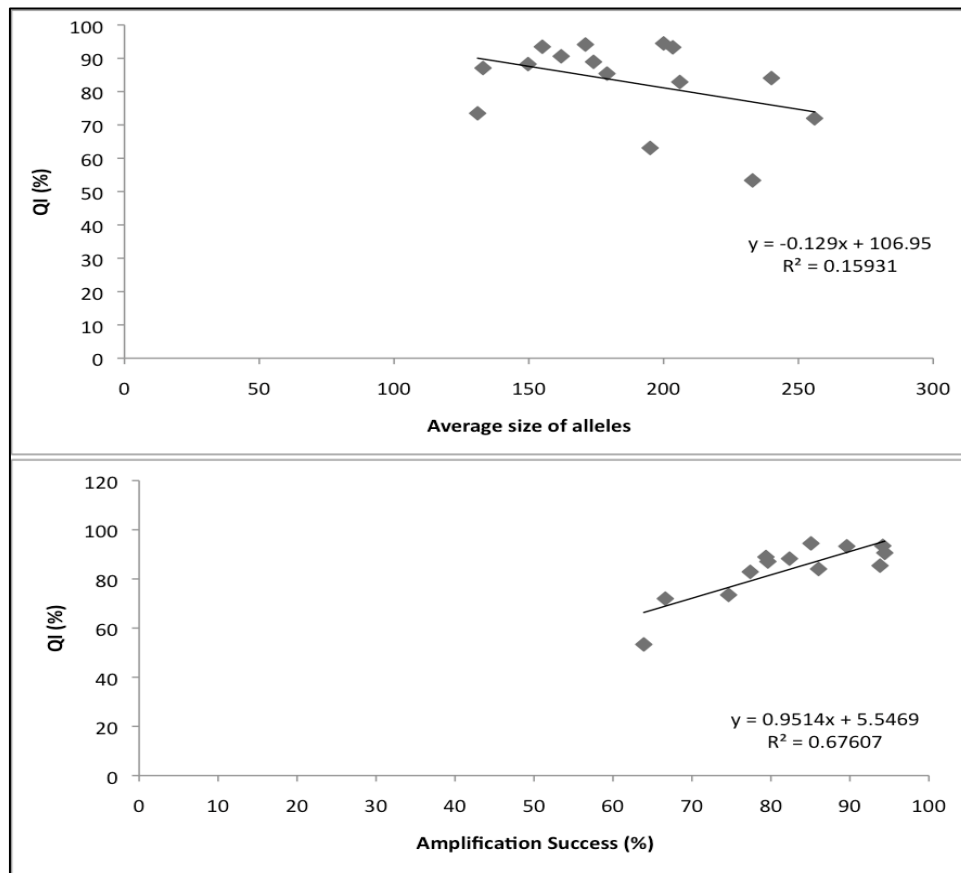


Figure 2.9: Average size of alleles (fragment length, bp) (top) and amplification success (%) (bottom) did not have a strong linear relationship with the reliability of genotypes (QI, %)

The average ADO rate across loci was of 15.6% (varying between 4.1% for locus D4S243 and 25.6% for locus D21S1442) and increased slightly with fragment size ($R^2 = 0.19$, Fig. 2.10), as previously noted by other studies (Buchan *et al.*, 2005; Broquet *et al.*, 2007). Allelic dropout rate appeared to be negatively correlated with amplification success (Fig. 2.10), a result also found by Buchan *et al.* (2005). The false allele rate across loci was of 2.98% (varying between 0.81% for D10S611 and 4.1% for D7S503).

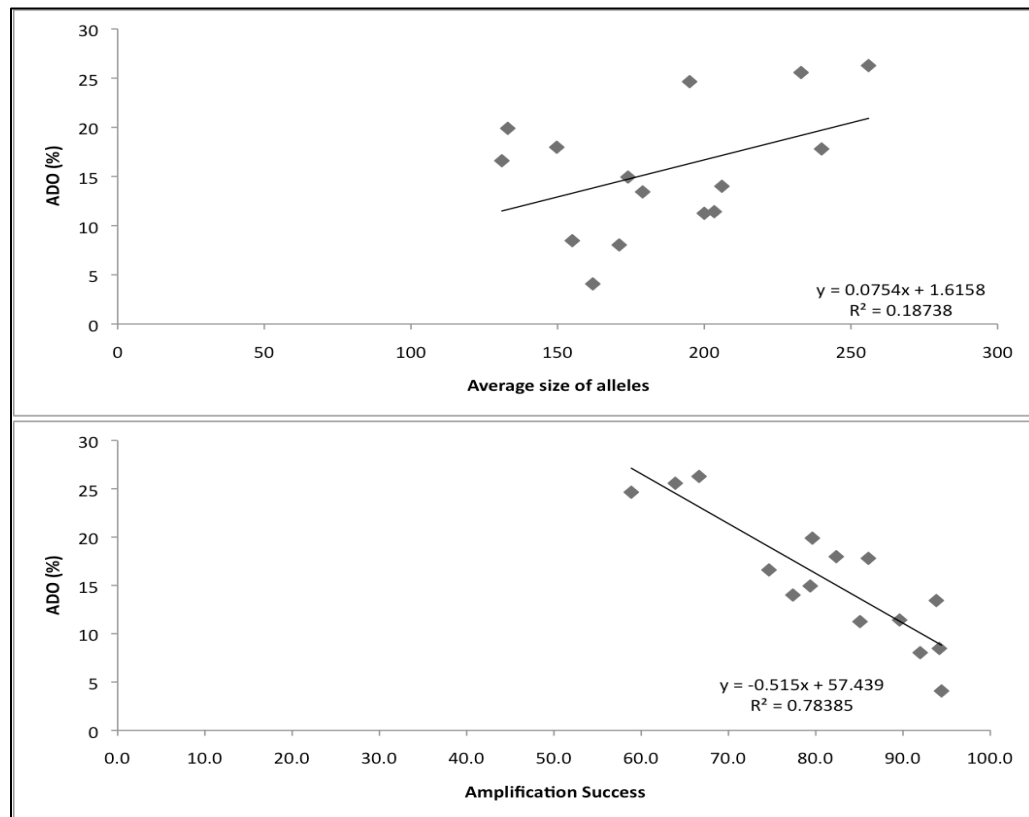


Figure 2.10: Allelic dropout rate (%) increased slightly with fragment size (bp) (Top) and is strongly negatively correlated with amplification success (%) (bottom)

Fickenscher (2010) and this study are not distinct in the average quality index of genotypes ($QI_{\text{Senegal}} = 84.2\%$ and $QI_{\text{GB}} = 84.4\%$), in the average allelic dropout rate across loci ($ADO_{\text{Senegal}} = 16.25\%$ and $ADO_{\text{GB}} = 14.99\%$) or in the average false allele rate across loci ($FA_{\text{Senegal}} = 4.74\%$ and $FA_{\text{GB}} = 2.90\%$). The main difference between studies seems to be related with difficulties in amplifying DNA. However, Guinea-Bissau samples showed higher ADO rates for D7S2204, D12S375 and D7S503 (13.5%, 9.7% and 7.3% difference between studies, respectively) and Senegalese samples show a higher ADO rates for D5S1457, D2S1326 and D4S243 (15.9%, 6.22% and 6.18% difference between studies, respectively).

Genotyping success (measured here as the proportion of samples reaching a consensus genotype per locus) varied across loci. On average a consensus genotype was achieved for 93.70% of samples per locus (database with 6.2% missing data). D3S1766 and D3S1768 were the ones with highest genotyping success (with 100% of samples reaching a consensus genotype) and locus D21S1442 showed the poorest results (only 73.62 % of samples were genotyped).

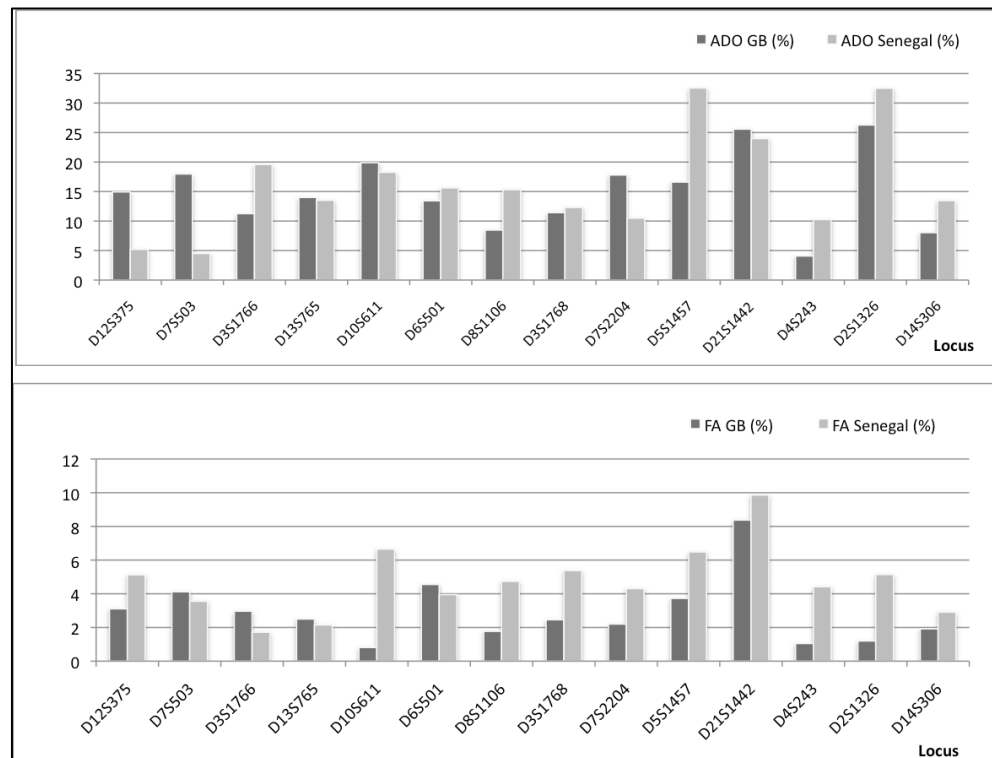


Figure 2.11: Guinea-Bissau and Senegalese samples (Fickenscher, 2010) are not distinct in the average allelic dropout rate (ADO, %) (Top) or false allele rate (FA, %) (bottom) across loci

2.3.2.3 – *Probability of Identity*

The probability that two individuals drawn at random from the population shared the same genotype at all fifteen microsatellite typed loci using (PIbiased) was of 3.4×10^{-11} or using a more conservative measure (PIsibs) was of 2.6×10^{-5} . PIsibs are the values that give high credibility to this analysis due to the sampling strategy of related individuals. D14S306 was the most informative to distinguish between individuals ($PI_{biased_D14S306} = 0.63 \times 10^{-2}$ and $PI_{sibs_D14S306} = 3.83 \times 10^{-1}$), followed by D21S1442 ($PI_{biased_D21S1442} = 8.35 \times 10^{-3}$ and $PI_{sibs_D21S1442} = 1.51 \times 10^{-1}$). The distinction of individuals is reliable with only 6 loci since the probability of identity approaches zero (see Fig. 2.12).

2.3.2.4 – *Summary diversity indices*

Mean number of alleles across the fifteen loci was of 5.1 alleles, varying between seven alleles (D7S503 and D21S1442) and three (D10S611). The most informative loci of this set were D21S1442, D4S243 and D14S306 (with a polymorphic information content of 72.0%, 66.5% and 74.0%, respectively) (Table 2.8).

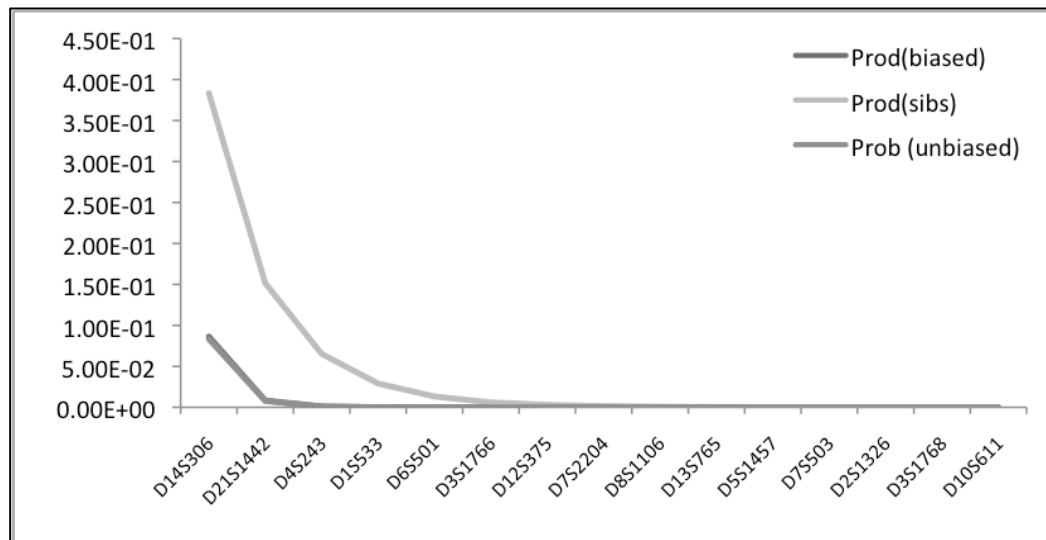


Figure 2.12: Distinction of individuals is reliable with only 6 loci when the cumulative probability of identity (PI sibs) approaches zero. Note that Prob Unbiased is the same as Prob (biased).

Average expected heterozygosity (H_e) across the fifteen loci typed was of 0.61, varying between 0.34 (D10S611) and 0.78 (D14S306). D4S243 and D21S1442 also presented rather high H_e values (0.70 and 0.76, respectively). Average observed heterozygosity (H_o) was of 0.56. D7S503 and D3S1768 presented a higher proportion of homozygotes (H_o 0.48 and 0.50, respectively) while D4S243 and D14S306 presented a higher proportion of heterozygotes (H_o of 0.73 and 0.70, respectively). D12S375, D6S501, D21S1442, D2S1326 and D14S306 exhibited significant deviation from proportions of Hardy-Weinberg equilibrium (HWE). However, after Bonferroni correction, only D6S501 and D21S1442 remained out of equilibrium (Table 2.8). Positive and relatively high fixation indices were observed for most loci, with the exception of D13S765, D6S501, D7S2204 and D4S243 (respectively -0.070, -0.007, -0.064, -0.045, respectively). D21S1442 presented an exceptionally high value of F_{is} (0.34) (see Table 2.8).

2.3.2.5 – Exclusion of locus D21S1442

Because D21S1442 showed the lowest amplification success and only 73.62% of samples achieved a consensus genotype, it had the lowest QI in combination with the highest ADO rate and a very high F_{is} of 0.34 (which suggests genotyping errors), this locus was excluded from the analysis. With the exclusion of locus D21S1442, the average ADO rate across loci decreased to 14.9% and the average FA rate across loci decreased to 2.49%.

Table 2.8: Summary diversity statistics for fifteen loci set used in this study: N (sample size); Na (number of different alleles); PIC (Polymorphic Information Content, %), Ho (Observed Heterozygosity) He (Expected Heterozygosity), *Fis* (Fixation Index). HW (Hardy-Weinberg equilibrium). Note that loci in HW non-conformity are in bold and significance accounts for multiple Bonferroni's corrections

Locus	N	Na	PIC	Ho	He	HW Equilibrium	After Bonferroni Correction	<i>Fis</i>
D12S375	161	6	57.1	0.57	0.61	p=0.033	NS	0.060
D7S503	162	7	49.0	0.48	0.54	p=0.055	NS	0.105
D3S1766	163	5	61.4	0.62	0.67	p=0.40	NS	0.077
D13S765	152	4	51.4	0.63	0.58	p=1.96	NS	-0.070
D10S611	159	3	30.6	0.29	0.34	p=0,07	NS	0.142
D6S501	161	5	61.4	0.68	0.67	p=0.021	p=0.0031	-0.007
D8S1106	162	4	52.8	0.58	0.59	p=0,63	NS	0.030
D3S1768	163	5	42.4	0.49	0.49	p=0.53	NS	0.004
D7S2204	156	6	52.9	0.64	0.60	p=0.51	NS	-0.064
D5S1457	140	4	47.8	0.51	0.55	p=0.64	NS	0.069
D21S1442	120	7	72.0	0.50	0.76	p=0.000	p=0.000	0.341
D4S243	161	6	66.5	0.73	0.70	p=0.31	NS	-0.045
D2S1326	140	4	47.0	0.47	0.54	p=0.01	NS	0.133
D14S306	161	6	74.0	0.70	0.78	p=0.04	NS	0.096
D1S533	130	5	62.0	0.56	0.69	p=0.054	NS	0.181

2.3.3 – Sex Determination

The sex determination protocol was relatively successful (with 71% amplification success) and results were highly reliable (QI of sex determination = 82.7%). Rates of allelic dropout and false alleles were rather low (ADO = 9.3% and FA = 1.2%). In the final dataset (including tissue samples) of 161 individuals, this protocol distinguished 95 females and 62 males (with 6 samples not achieving any result).

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Chapter 3 Size matters in the hunting practices and bushmeat trade for Guinea baboons (*Papio hamadryas papio*) in Guinea-Bissau

“Are you a hunter?”

I am a hunter, a fisherman and married”

(Guinean-Bissau hunter interviewed about his occupation)

Size matters in the hunting practices and bushmeat trade for Guinea baboons (*Papio hamadryas papio*) in Guinea-Bissau

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3.1 - Abstract

The guinea baboon, the vervet monkey and the colobus monkeys (*Procolobus badius* and *Colobus polykomos*) are thought to be frequently targeted species in the bushmeat trade in Guinea-Bissau (GB). For Guinea baboons, these practices have contributed to a range contraction of the distribution within the country. In this study, we aimed to describe the hunting practices towards Guinea baboons and to estimate the quantities of baboon carcasses sold at specialized meat markets. By following the trade at two bushmeat markets in Bissau during 19 days and across four months, we found 150 carcasses from six primate species being sold. The trade reached 17 specimens/day and decreased towards the beginning of the rainy season. Male baboon carcasses were sold at a price 60% higher than any other primate due to their larger body mass. We used DNA barcoding approach (*Cytochrome c Oxidase subunit I* and 12S rRNA mitochondrial DNA fragment) to assess the accuracy of morphological identification performed at the markets. We found that the vervet monkey and the Campbell's monkey were the most frequently traded species (32.2 % and 30.6 %, respectively) followed by *P. h. papio* (19.4%). Most of the misidentifications occurred between species of similar body weight and *P. h. papio* showed a relatively lower error rate. Seven semi-structured interviews conducted with hunters revealed a preference towards male baboons. The current increased difficulty in finding this primate suggests a recent population decline or behavioural changes to avoid detection by hunters. We emphasize the importance of combining different approaches (molecular identification and qualitative information) when evaluating the bushmeat trade in primate species.

Keywords: Guinea baboon, Bushmeat, Guinea-Bissau, Barcoding, qualitative information

3.2 - Introduction

Many African mammals are currently intensively hunted at globally significant rates (Fa and Brown, 2009; Fa *et al.*, 2000; Milner-Gullanda *et al.*, 2003). Wild meat is often regarded as an important source of animal protein in West African rural areas (van Vliet, 2011). However, over the last decades and at many locations across West Africa, hunting activities have changed from a subsistence pursuit to an organized activity with strong commercial drivers (Chapman *et al.*, 2006; Fa and Brown, 2009; Refisch and Koné, 2005; van Vliet, 2011). Such a shift has been explained by the multiplication of consumers in a regional and international scale (Chaber *et al.*, 2010; Fa and Brown, 2009; Mittermeier, 1987; Nijman *et al.*, 2011; Starin, 1989).

Commonly, the bushmeat trade remains obscure and is not included in economic figures (Bowen-Jones, 1998; van Vliet, 2011) but it can represent a significant fraction of the West-African countries' gross domestic product (Bowen Jones and Pendry, 1999; Pailler, 2005). The bushmeat trade can be highly structured and includes hundreds of stakeholders (Mendelson *et al.*, 2003; Refisch and Koné, 2005; van Vliet, 2011) and their number and relative importance varies across countries (e.g. Mendelson *et al.* 2003; Refisch and Koné 2005). The supply chain encompasses the hunters (usually men), the transporters or intermediate traders and bushmeat market vendors and/or urban restaurant owners (who are often women) (Crookes and Milner-Gulland 2006; Mendelson *et al.* 2003; Pailler 2005; van Vliet, 2011 2011). The depletion of wildlife threatens not only the biodiversity of the ecosystems but also the daily life of those is involved in this trade (Milner-Gullanda *et al.*, 2003). For remote villagers with limited income alternatives or living in a poor and politically unstable country, this income is very important (Bowen Jones and Pendry, 1999; de Merode *et al.*, 2004; Mendelson *et al.*, 2003; van Vliet, 2011). It can be used on vital daily commodities (e.g. medicines, fishing nets, school fees) (de Merode *et al.*, 2004) or in less indispensable items (e.g. radios, modern clothes, cigarettes) (Casanova and Sousa, 2007; Coad *et al.*, 2010).

At the bushmeat markets, primates, ungulates and rodents are frequently consumed (Fa and Brown, 2009). Although ungulate and rodents species correspond to the largest biomass hunted, primate carcasses can still represent up to 20% of trade at

dedicated markets (Bowen-Jones, 1998; Fa *et al.*, 2006). Carcasses' cost is frequently determined by the animal's body mass, regardless of the taxa being traded and the condition of the meat (Wilkie *et al.*, 2005; Macdonald *et al.*, 2011). However, under conditions of species-limitation and depending on the freshness of the meat, this relationship is expected to be more complex (Albrechtsen *et al.*, 2007).

Both habitat loss and local hunting-driven declines (Fa *et al.*, 2000; Topp-Jørgensen *et al.*, 2009) contribute to the abrupt extinctions of primate populations (Barnes, 2002). Primate species are especially vulnerable (Chapman *et al.*, 2006; Fa and Brown, 2009) because: i) their sometimes large social groups and frequent vocalizations increase their exposure to hunters; ii) the use of shared sleeping sites allow for the hunting of several individuals with low effort and iii) their behavioural resilience allows for primates to remain at considerable densities after more sensitive species have disappeared (Fa *et al.*, 2005). Additionally, the primate species slow-reproductive features prevents a fast recovery for hunted populations (Fa and Brown, 2009) and they can be considered highly palatable by bushmeat consumers or may possess valuable physical features (e.g. skins) (Mittermeier, 1987; Struhsaker, 1999).

Medium to large bodied size species frequently become overharvested first (Bennett *et al.*, 2002; Peres 2000; Topp-Jørgensen *et al.*, 2009) because commonly, the hunters attempt to boost profit per unit of effort (e.g. time, number of cartridges, number of traps). Large-bodied species will usually be targeted first, regardless of frequency of encounters and rarity (Bennett *et al.*, 2002; Wilkie *et al.*, 2005). However, species-specific hunting rates may be conditional on the interactions between species biology, past or present abundance (Cowlshaw *et al.*, 2005; Albrechtsen *et al.*, 2007; Topp-Jørgensen *et al.*, 2009) and the hunter's decisions and behaviour (Fa and Brown, 2009). Intrinsic factors such as the preference for a given species, hunting propensity or technique and economic motivation can therefore affect species persistence (Mittermeier, 1987; Fa and Brown, 2009).

In Guinea-Bissau (GB) West Africa, previous reports claimed that large quantities of primates have been hunted to be sold in the capital (Bissau) or in other important urban areas (e.g. Buba, Quebo, Empada, Catió, Bambadinca and Bedanda) (Cá, 2008; Casanova and Sousa, 2007). Even though crop-raiding conflicts or subsistence hunting practices play a role (Costa, 2010), primate disappearances seem to

be related to hunting practices by professional hunters and militia (Casanova and Sousa, 2007) and with severe loss of habitat (Gippoliti and Dell’Omo, 2003).

The most targeted species are thought to be the Guinea baboon (*Papio hamadryas papio*), the Vervet monkey (*Chlorocebus sabaeus*) and the Colobus monkeys (*Procolobus badius* and *Colobus polykomos*) (Cá, 2008; Casanova and Sousa, 2007). Hunters have been assumed to prefer male baboons as these carcasses reach twice the price of other species (e.g. Vervet monkeys) in the intermediate trade (Cá, 2008; Casanova and Sousa, 2007). Additionally, it is common to observe young baboons being kept as pets throughout the country (Casanova and Sousa, 2007; Ferreira da Silva personal observation; Hockings and Sousa, 2011). The demographic consequence of such practices for Guinea baboons is a significant range contraction within GB (Casanova and Sousa, 2007; Gippoliti and Dell’Omo, 2003).

Nevertheless, characterization of GB hunting activities and the bushmeat trade is still deficient. Hunting activities in GB are illegal and locals, hunters or vendors are usually hesitant to provide information on such a delicate subject (Costa, 2010). Furthermore, morphological identification of the carcasses is hindered. In GB, as in other locations in West Africa (Bowen Jones and Pendry, 1999; Bowen-Jones, 1998), hunters smoke carcasses to aid the preservation of the meat before consumption (Cá, 2008; Casanova and Sousa, 2007), which restricts the distinction between species with similar size and body shape.

Here, we aimed to describe the hunting practices towards Guinea baboons in Guinea-Bissau and to estimate the quantities of baboon carcasses sold at specialized meat markets. We followed the trade at two markets in Bissau across four months and used a DNA barcoding approach (Hebert *et al.*, 2003) (mitochondrial DNA *Cytochrome c Oxidase subunit I* and 12S rRNA fragment) to test the accuracy of morphological identification performed at the markets (Teletchea *et al.*, 2005). As we were interested in the rationale behind the hunting behaviour and species-specific preferences, we conducted semi-structured interviews with hunters. Qualitative information cannot be generalized for the group under study as it focuses on few informants (Ritchie, 2005; Rubin and Rubin, 2004). Nevertheless, describing the diversity of opinions of individuals or sub-groups can complement and improve the interpretation of qualitative data (Ritchie, 2005).

3.3 – Methods

3.3.1 – Study Area

Guinea-Bissau (10°59'- 12°20'N and 13°40'-16°43'W), located in West Africa, is a small country (36,125 Km²) formed by a continental mainland and an archipelago (Bijagós islands). The region shows great heterogeneity in flora and vegetation (Catarino *et al.*, 2001) and a great level of faunistic diversity can still be found, including 11 species of primate species (Gippoliti and Dell’Omo 2003). Guinea-Bissau is considered one of the poorest countries in the world, where more than 68% of the population live below the poverty line (2002¹). Its GDP per capita in 2010 was of 161 US\$⁵. Southern GB (Quinara and Tombali) features the poorest areas within the country [more than 69% live with less than 2 dollars per day (PNUD 2006)]. Most of the population live in rural areas, dependent on subsistence agricultural and fishing activities (DGP, 2002) and on the country’s natural resources (DGP, 2002; United Nations, 1997; World Bank, 2004). Cash is scarce in rural areas and villagers usually exchange agricultural products for other goods (Forrest, 2003).

3.3.2 – Bushmeat trade

We followed the trade at two markets at the capital, Bissau (“Chapa” and “Rampa”). The markets were visited during nineteen days across four months. Visits were conducted in three different periods: from 1st to 5th March (five days); 30th March to 10th April (five days) and from 19th May to 6th June (nine days) in 2010. Visits took no more than fifteen minutes to avoid hindering the normal functioning of the trade. We counted the number of primate carcasses visible and recorded the morphological identification provided by the traders (Table 1, Appendix 3). We inquired price and origin. We also visited restaurants where primate bushmeat is consumed to ascertain the price of the dish.

⁵ <http://data.worldbank.org/country/guinea-bissau>

3.3.2.1 - *Molecular Identification*

We confirmed that primate carcasses arrive to the markets whole, charred and disembowelled (see Fig. 3.1). We collected 50 tissue samples. Samples were stored in tubes with 99% ANALAR ethanol and labelled with the morphological identification provided by the traders. Collection of unburned tissue (from the inside part of carcasses) was made wherever possible.

3.3.2.1.1 - *DNA Extraction, PCR amplification and sequencing*

Whole genomic DNA was extracted using DNeasy Blood and tissue kit (Qiagen©) following the manufacturer's protocol but allowing overnight lysis of the tissues. We amplified a 623 base pair (bp) fragment from the standard barcode region of the *Cytochrome c Oxidase subunit 1* (COI) of the mitochondrial DNA (mtDNA). We used the three primer sets used in Lorenz *et al.*, (2005) and Folmer *et al.*, (1994): "OWMCOI", "VERTCOI" and "FOLMER" (see Table 2, Appendix 3). We followed a sequential amplification when no PCR product was evident starting by the "OWMCOI" set, followed by the "VERTCOI" set and ending with the "FOLMER" primer set. As we could not find any sequence identified as *Cercopithecus campbelli* in GenBank, we identified this species using a fragment of the mtDNA 12S rRNA gene. We used a set of primers previously designed by Kessing *et al.*, (1989) and modified by Kocher *et al.*, (1989):

AAACTAGGATTAGATACCCTATTAT,	12SbiR
AAGAGCGACGGGCGATGTGT to amplify a 386bp fragment.	

MtDNA fragments were amplified in 20µl using 10µl Qiagen © Master Mix with 2µl of template DNA and using 2µM final concentration of the primers. The thermal cycling programme started at 95°C for 15 min, 40 cycles of 30 sec at 94°C, 90 sec at 50°C (for all primers sets) and 90 sec extension at 72°C. A final extension step of 10 min at 72°C was included. Thermal cycling was performed on an Applied Biosystems GeneAmp PCR System 9700. PCR products were purified with 10 units of Exonuclease I and 5 units of Antarctic Phosphatase (New England Biolabs). Purification cycling programme started at 37°C for 30 min, 80°C for 20 min and finished at 12°C for 5 min. Samples were sequenced bidirectionally using MacroGen Europe's EZ-seq direct service. A consensus between the forward and reverse sequences for each sample was made by visual comparison. All sequences were manually aligned using BioEdit version 7.0.5.3

(Hall, 1999). Sequences were checked for the presence of nuclear copies by assessing frameshift mutations (insertions or deletions), the number of bands on gels, or unlikely phylogenetic placement in trees [following Bensasson *et al.*, (2001) and Song *et al.*, (2008)]. No evidence of the presence of nuclear copies was found.

To assign each COI sequence to its respective species we followed Frézal and Leblois (2008) method: 1) we searched in databases for the most similar sequences; 2) we chose the most similar sequences as “voucher” examples; and finally 3) we used a phylogenetic approach (using genetic distances) to group the samples to the vouchers sequences. Each query sequence was compared to all COI reference sequences on Inprimat⁶ and NCBI⁷ databases. We included in the alignment sequences from *Erythrocebus patas* (E PATAS 1, accession number EF568610.1 and E PATAS 2, accession number AY972702.1), *Chlorocebus sabaeus* (C SABAEUS 1, accession number EF597503.1 and C SABAEUS 2, accession number NC_008066.1) and *Papio hamadryas papio* (P PAPIO 1, accession number AY972684.1 and P PAPIO 2, accession number AY972678.1) found on both Inprimat and NCBI. We used two vouchers for *Procolobus badius*: a sequence found only in NCBI (P BADIUS 1, accession number NC_008219.1) along with a faecal sample collected and extracted by T. Minhós from a visually confirmed *P. badius* (P BADIUS 2). We included in the alignment *Colobus polykomos* voucher samples (C POLYKOMOS 1, accession number AB016731.1 and C POLYKOMOS 2, accession number AY972692.1). For *Cercopithecus campbelli*, we sequenced a fragment of the 12S rRNA gene for all samples suspected to be *C. campbelli* and compared it with two *C. campbelli* sequences (accession numbers AY665618.1 and AY665619.1) to confirm specific identification.

Using Mega v5.01 (Tamura *et al.*, 2011) we constructed a Neighbour joining (NJ) tree to view clustering of sequences with bootstrap support (1,000 replicates). We used the Kimura 2-parameter model, including both transitions and transversions.

3.3.2.1.2 - Specific morphologic identification error and projection of trade

We calculated the error rate associated with the morphological identification for each species. Species-specific error rate was defined as the frequency of molecular

⁶ www.inprimat.org

⁷ <http://www.ncbi.nlm.nih.gov/genbank/>

identifications that did not correspond to the morphological identifications registered at the markets. The number of individuals traded is presented as a range, varying between the i) minimum numbers of individuals molecularly assigned to that species and between the ii) maximum numbers of individuals potentially being traded.

To estimate the potential maximum number of individuals at the markets for each species, we summed the true positive identification frequency (A) with the false negative identification frequency (B). Therefore for any species:

$$A = NM \times (TL / TS)$$

NM - Number of morphological records; TL – number of tissue samples identified morphologically by the sellers and molecularly assigned to that species; TS – number of tissue samples collected for a species and labelled as morphologically identified by the sellers.

$$B = IM \times (TNL / NSOS)$$

IM - Number of individuals in both markets which not identified as that species; TNL - Tissue samples molecularly assigned to the species *but not* identified morphologically by the sellers as that species; NSOS - Number of tissue samples collected at both markets that were morphologically identified by the sellers as that species

(For more details on calculations and examples see Table 2, Appendix 3)

We calculated the relative percentage of each traded species using the potential maximum number of individuals being traded per species. We then extrapolated the trade for the entire dry season (November to May, 212 days) using the relative percentages of trade for each species.

3.3.3 – Interviews with Hunters

Seven semi-structured interviews were conducted in Cantanhez Woodlands National Park and in Cufada Lagoons Natural Park (located within Tombali and Quínara regions). The hunters were contacted using a snowball approach e.g. the interviewee were questioned about who else besides themselves might constitute a good informant to describe hunting practices (Cohen and Crabtree, 2006; Moreira, 1994). We contacted the interviewees at their houses, where the interviews were taken and recorded. The purpose of the study was described and the interviewees were able to remain anonymous. It was guaranteed that the interviewees and their activities would not be reported to the local authorities.

Interviews were conducted in Creole, with the exception of two (in Portuguese and in Sosso, translated to Creole by a park guide). The interviews were private, with the exception of two, which were conducted in the company of an informant. When a different currency was referred during the interviews, a conversion for West African CFA franc (FCA) was requested (in this study, one US dollar (\$) corresponds to 542 FCA).

To avoid incriminating questions about current hunting practices, most of the questions focussed in past activities (before the establishment of the parks) or on other hunter's activities. Nevertheless, the two interviewees that admitted to hunt primates answered direct questions. The questions were constructed to first address broad concepts (e.g. Which animals are hunted? Which primates are hunted?) and subsequently, species-specific questions (e.g. What can you tell me about the hunting of baboons?). The script of the interviews started with questions focussing in the life of the interviewed (age, place of birth, number of family elements, main daily activities) and then focussed on hunting practices (species, experiences and behaviour, weapon or trap type used, hunting areas, trade prices). If not referred to, we asked about hunted primate species. Additionally, we enquired about which species were seen in the past. From those, it was inquired which species are least detected now and why. Finally, opinions about the functioning of the parks were addressed.

The interviews were transcribed verbatim and only the relevant information was organized into the following categories (Rubin and Rubin, 2004):

- i) Description of hunting practices (hunters experience, type of weapons, instructor, hunting places and techniques);
- ii) Hunted species:
 - a. List of hunted species;
 - b. Uses of hunted species:
 - i. Uses:
 - 1. Meat trade (quantities and prices and in the appropriate cases, procedures after hunting and use of cash);
 - 2. Pet trade (species, prices, costumers, locations and hunting techniques)

- iii) Species abundance (list of species seen in the past and hunter's classification of their current abundance);
- iv) Perception on animals (when referred);
- v) Opinions about park management.

The interviews were read and re-read to capture the context in which the information was referred (Rubin and Rubin, 2004).

3.4 - Results

3.4.1 – Bushmeat trade at Bissau

During 19 days, 150 primate carcasses were counted at the bushmeat markets (Fig. 3.1). Along with baboons, we identified five other primate species being traded: *Procolobus badius* (Western Red Colobus), *Colobus polykomos* (Western Black-and-White Colobus), *Cercopithecus campbelli* (Campbell's Monkey), *Chlorocebus sabaeus* (Green Monkey) and *Erythrocebus patas* (Patas monkey). Non-primate species observed at the markets included the Greater Cane Rat (*Thryonomys swinderianus*), the Crested Porcupine (*Hystrix cristata*), pangolins (*Manidae sp.*), the Red River Hog (*Potamochoerus porcus*) and duikers (*Cephalophus sp.*).



Figure 3.1: Primate carcasses arrive to the markets whole, charred and disembowelled

More primates were sold at Chapa (113) than Rampa market (37). More primates were traded in the second period (17 carcasses/day), followed by the first period (10 carcasses/day) (Fig. 3.2). In the third period of the study (19th May to 6th June), approaching the start of the rainy season, we found a sharp decrease activity (only 2 primates per day in Chapa market and no trade at Rampa market) (Fig. 3.2). On average (\pm SD) we found 9.5 ± 7.4 primates/day were being traded at both markets.

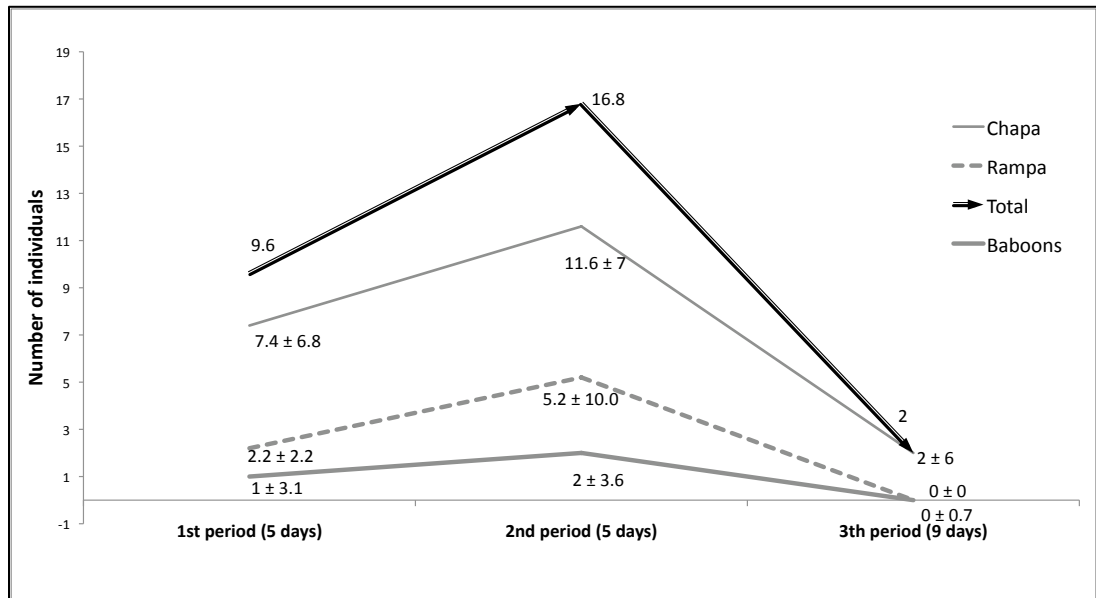


Figure 3.2: The daily average of individuals found at the markets (Chapa and Rampa) (\pm SD) and total for both markets in each period of the study (1st period: 1st to 5th March; 2nd period: 30th March to 10th April; 3rd period: 19th March to 6th June). Note that the second period registered a peak in the trade while in the third period, the trade decreased

We counted at the markets 26 putative baboons. The maximum daily average of two baboons/day was reached in the second period (Fig. 3.2). The highest proportion of baboon's carcasses (83%) was sold during a shorter period, between 4th March and the 31st March (Fig. 3.3).

At the markets, the price varied with respect to carcass size because weighting instruments were not available at the stands. *P. h. papio* males were the most expensive. Male baboons were sold between 10,000 and 15,000 CFA (approximately US\$ 19 and US\$ 40). Female baboons were being sold at the same price as other species, between 4,000 and 8,000 CFA (approximately US\$ 8 - US\$ 16). Carcass origin was mostly referred to be from the “south” of GB. The origin of baboon carcasses was referred to be Cossé, Xitole (Bafatá region, central GB) and Cacine (Tombali, Southern GB).

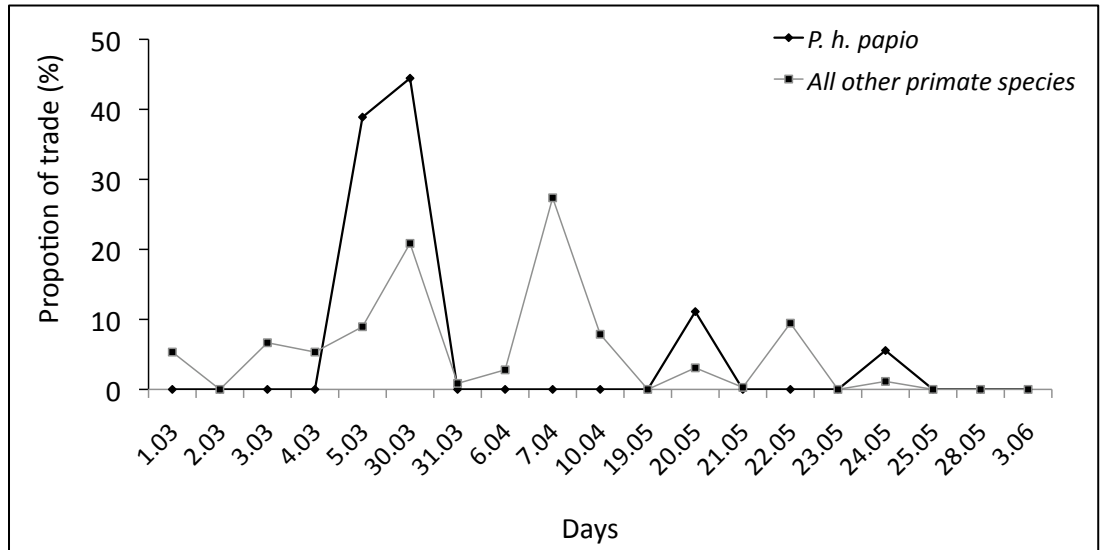


Figure 3.3: Daily proportion of trade (%) for *P. h. papio* and for all other primate species combined along the days (day.month) of study. Note that the peak of Guinea baboon carcasses trade was between 4th March and the 31st March.

At the restaurants, we found the primate meat being sold and consumed as a snack whilst drinking alcohol. The meal, consisting of four primate meat pieces cooked in a stew, cost approximately 1,250 CFA (approximately US\$ 2.62). Customers were found to greatly prefer hands, feet and heads. We were able to register one meal being cooked (Fig. 3.4) that consisted of two heads (female baboon and a putative green monkey head) and several pieces of meat.



Figure 3.4: Preparation of primate meat stew at a specialized restaurant (these restaurants are locally called *Abafatórios*). The white circle shows a baboon head.

3.4.1.1 - Molecular assignment

All tissue samples were successfully extracted and sequenced for both mtDNA fragments. We sequenced 46 samples with the OWMCOI primer set, two samples with the VERTCOI primers and the two samples using the FOLMER primer set. All sequences were identified using BLASTn (Altschul *et al.*, 1990) and compared with NCBI database *COI* sequences. Across species and discounting *Cercopithecus campbelli*, we obtained a 0-6% range of dissimilarity. Using the 12S rRNA fragment, all putative *Cercopithecus campbelli* samples showed 2% dissimilarity with *C. campbelli* vouchers AY665619.1 and AY665618.1.

In the NJ tree, all *COI* sequences grouped in six highly supported clusters (100% of the bootstrap support) (Fig. 3.5). In the tree, samples are labelled with the morphological identification provided by the sellers but are grouped accordingly to the genetic similarity with vouchers retrieved from the databases and with the other bushmeat samples. The six species referred to by the sellers at the markets (*C. campbelli*, *P. badius*, *C. polykomos*, *P. h. papio*, *E. patas* and *C. sabaesus*) were sampled and we did not find additional primate species at the markets. The 12S rRNA tree did not show such highly supported clades but all sequences belonging to clade 3 in Fig. 3.5 (putative *C. campbelli* sequences) were grouped together in the same clade along with the vouchers AY665619.1 and AY665618.1 (Fig. 3.6).

On average, the morphological identification error rate across species was 23.4%. *P. h. papio* had one of the smallest error rates (8%), in contrast with *C. campbelli*, *P. badius* and *C. polykomos*, which had the highest error rates (59%, 40% and 33%, respectively). We did not find any error in the morphological identification of *E. patas* and *C. sabaesus*. Two samples identified morphologically as baboons were molecularly identified as being *C. campbelli* and *E. patas* (Fig. 3.5). *C. campbelli* was mainly confused with *C. sabaesus* (nearly 85% of misidentifications) and with *P. badius* (8%). *P. badius* was also most frequently confused with *C. sabaesus* (75%) and with *C. campbelli* (25%).



Figure 3.5: COI neighbour-joining tree (based on 1000 replicates) calculated from Kimura 2-parameter genetic distance. The six highly supported clusters (100 bootstraps), labelled in the figure, correspond to the six species referred to the markets by the sellers: 1 - *C. sabaeus*; 2 - *E. patas*; 3 - *C.*

campbelli, 4 - *P. h. papio*; 5 - *C. polykomos*; 6 - *P. badius*. The labelling of the samples in the tree reflects the morphological identification at the markets. Arrows point to the misidentification cases for baboons

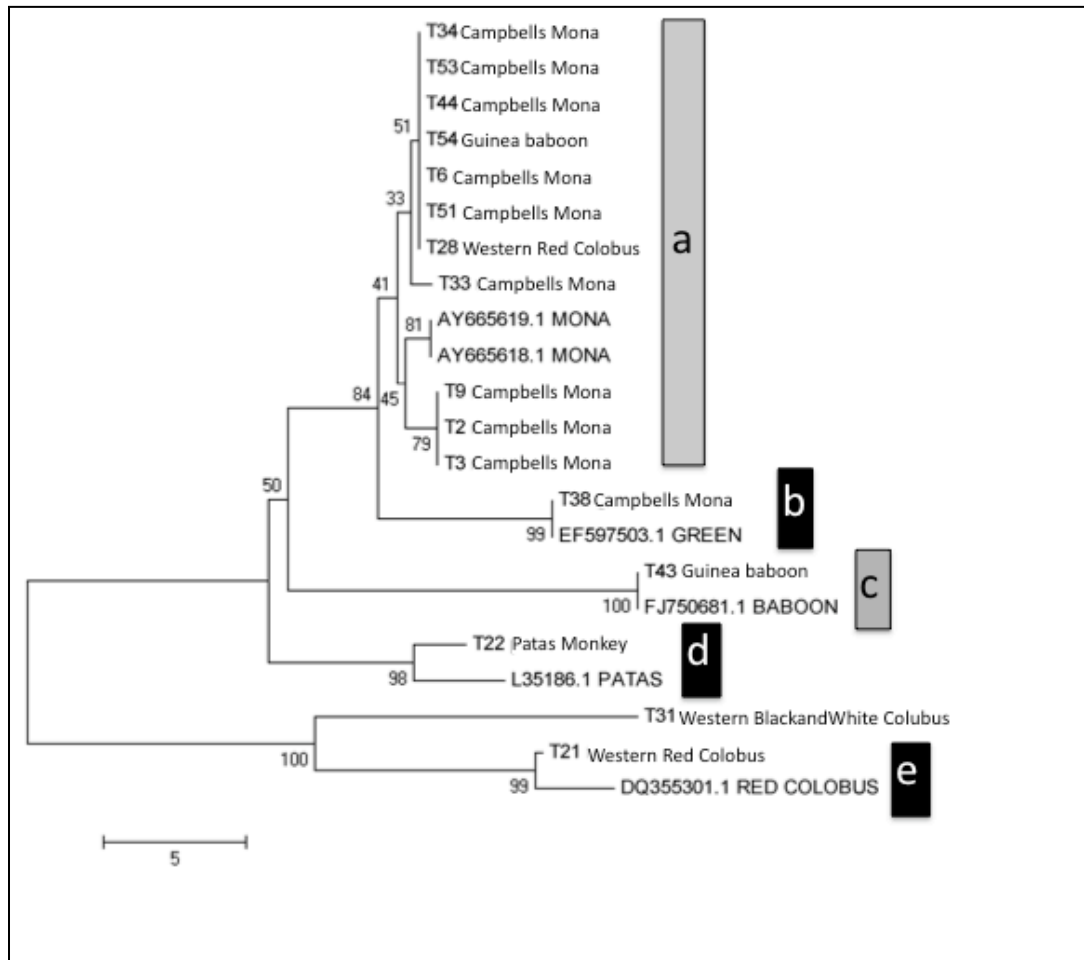


Figure 3.6: 12S rRNA neighbouring-joining tree (based on 1000 replicates) calculated from Kimura 2-parameter genetic distance. *C. campbelli* clade is labelled in the figure (a). All sequences grouped in clade 3 in Fig. 3.5 (putative *C. campbelli* sequences) grouped in the tree with voucher AY665619.1 and AY665618.1. The other clades represent the species included in the 12S rRNA alignment: b - *C. sabaesus*; c - *P. h. papio*; d - *E. patas*; e - *P. badius*. The labelling of the samples in the tree reflects the morphological identification at the markets

By applying the correction factor to the total number of specimens observed at the markets, *C. campbelli* decreased by 35 specimens but all other species increased in number (see Fig. 3.7). *P. h. papio* increased by 13 specimens, reaching a maximum of 36 animals (see fig. 3.7).

After correcting the morphological identification (Fig. 3.7), we estimated that *C. sabaesus* and *C. campbelli* were more the most frequently traded (32.2 % and 30.6 %, respectively) than any other species, followed by *P. h. papio* (19.26%). *P. badius* (11.5%), *E. patas* (4.9%), and *C. polykomos* (1.4%) were the least traded. We extrapolated the number of primates traded in Bissau over the entire dry season (212

days). Using the average across the three periods of daily trade, we estimated 2008 primates being traded along the dry season. Assuming the species-relative percentages estimated by this study, we predicted that 646 specimens of *C. sabaesus*, 616 of *C. campbelli*, 387 specimens of *P. h. papio*, 232 specimens of *P. badius*, 99 specimens of *E. patas* and 28 specimens of *C. polykomos* are traded in the bushmeat markets in Bissau.

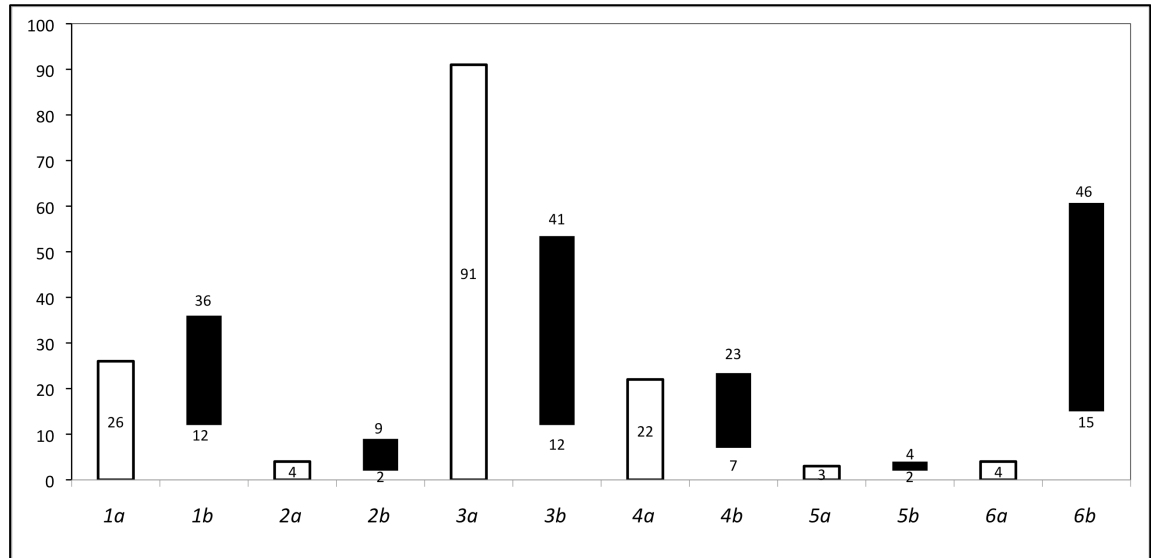


Figure 3.7: Total number of specimens per species. White bars refer to the morphological identification registered at the markets (a). Values inside white bars refer to the specimens identified morphologically. Black bars represent the potential range of animals being traded after correction by molecular identification (b). Values below black bars refer to the minimum number of specimens per species (i.e., samples molecularly assigned to that species). Values above black bars represent the potential maximum number of individuals at the markets. 1 - *P. h. papio*, 2 - *E. patas*, 3 - *C. campbelli*, 4 - *P. badius*, 5 - *C. polykomos*, 6 - *C. sabaesus*.

3.4.2 – Semi-structured interviews with hunters

The hunters addressed were mainly between 40 and 65 years old, but one was much younger (ID #3, 34 years old) and one was much older (ID #1, 99 years old). With the exception of interviewee ID #5 and ID #6, the interviewee's birthplace did not correspond to the place where they were living at the time of the interview. All were married and responsible for sustaining a large (and poor) family. They did not subsist exclusively from hunting activities; they defined their activities to vary between farming or fishing and hunting: "I do not fish there. I am a farmer. This is my work. I grow rice, peanuts and cassava. But that year, I spent a lot of money with this work [fishing] (...) I asked them if I could help them fishing. But, in other conditions, I would not fish, I would just farm" (ID #4) or "I am hunter, fisherman and married" (ID #7). However, one interviewee defined himself as a hunter: "My job is to hunt. (...) When my father became old, I became a

hunter” (ID #6); and another described his main activity as a hunting guide (for foreigners).

All of them started to hunt at a young age (15 years old) and most of them have been hunting for more than forty years. This skill was learned from their fathers [*“He learned from his father (...) His father hunted elephants”* (ID #1)], with other elements of their family [*“my grandmother, my father, their job was to hunt big animals”* (ID #3)], or with other hunters living in the same community. One interviewee learnt to shoot during the war of independence in a Portuguese army’s headquarter: *“Who taught you how to hunt?” “Alves, he was a captain. Each week, I used 55 bullets to train (...) I learned in 3 years. When the war was over I continued to hunt”* (ID #5).

During their lifetime, they used several types of weapons: shotguns (12 gauge ammunition shotgun, locally named “calibre 12”) or craftsman guns (handmade by blacksmiths). During war, as two interviewees admitted, they hunted with “mausers”, “automatic rifles” or “Kalashnikov” rifles: *“I was hunting a lot. Once, with someone, I went to hunt with an AKA (AKA 47 or Kalashnikov). I shot four buffalos”* (ID #4); *“Alves asked (...) for me to have a weapon. But I said to him that a Mauser was enough because I could hunt birds, any animals (...). A Mauser could shoot at more than 100 meters; a shotgun does not have the same strength”* (ID #5). Most of them owned their hunting guns. Craftsmen’s guns were considerably cheaper than shotguns: *“[craftsmen’s guns] Between 25,000 and 40,000 CFA without the card [approximately US\$ 47.5] because it doesn’t come with a warranty” (...). If it is from white people [12 gauge ammunition shotgun], it costs 130,000, plus the card, 150,000 CFA in total”* [approximately US\$ 206] (ID #5). One referred that he would borrow one shotgun from colleagues when necessary because the park guards took his shotgun away: *“I don’t have a gun now, they took it because of a monkey”* (ID #3).

The hunting areas referred to during the interviews were spatially spread across GB. The places referred included not only the parks but also villages located in the northern, central and southern GB, the Bijagós islands and the Boé region (see Fig. 3.8). Hunting locations seem to be determined by the presence of certain species and constrained by agents acting against hunting practices. In their words, *“Barraca de Nino [located in Cufada] it is a way to find big animals”* (ID #6) or *“(…) I would go to the margins of salty water because baboons are usually there than in other places”* (ID #3); *“we would go there to get the hippos, where the hippos crossed. (...) we would get a boat, we killed one and another and came back to Bissau”* (ID #2); *“I am going outside Cufada. I am going to Mampata*

(...). Sometimes, if you want to hunt a big animal, you need to hunt outside the park to have less problems” (ID #3).

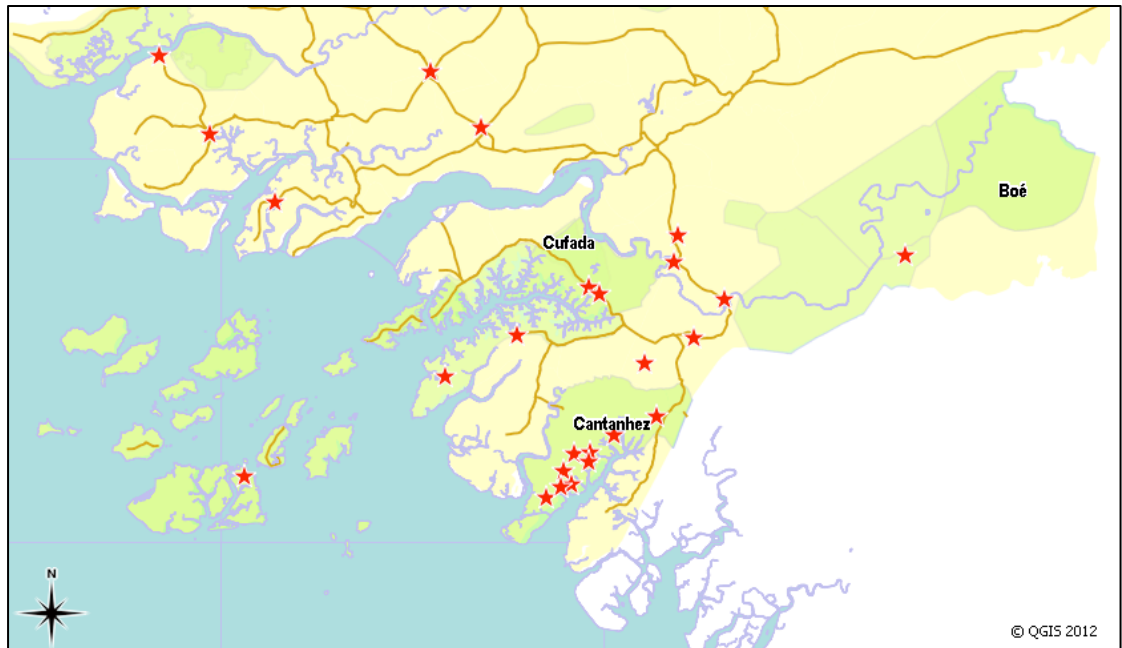


Figure 3.8: Hunting areas referred to by the hunters during the interviews. Stars point to locations referred to by the interviewees. The main roads connecting to the capital are represented by a continuous brown line. Protected areas are indicated in green.

The hunted species referred to by the interviewees (e.g. including those hunted by the interviewee and the species hunted by others), the hunting technique and the use of the carcasses are resumed in Table 3.1 and 3.2. It is worth noting that, in most cases, large sized animals (such as buffalo, gazelles and in one case, elephant) were referred to before the smaller ones (such as monkeys). Big animals were associated with memorable events: “Because I killed three leopards during the war” (ID #2); “I killed 36 maxwell duikers and one leopard” (ID #3) or “During the war period, my mestra [a woman] that was in Cachincha came here to kill a leopard” (ID #1); “Here, there were plenty of elephants, my father killed one” (ID #1).

Buffalos were regarded as large animals: “One leg weights around 50 kg, a head around 30-40 kg” (ID #6), “This huge animal with horns”(ID #5) and able to provide for a great number of people: “during that time, hunting a big animal could allow many people to eat. One buffalo would be enough for one village for example”(ID #6). Buffalo population in GB were thought to be abundant in the past: “when he went to the forest to hunt buffalos, he found one that was not that fat, he left that one and kept on searching” (ID #1); “It was easy [to see buffalos] at that time. In Cafatché, I killed 2 buffalos there” (ID #5). One interviewee (ID

#6) claimed that his father killed 99 buffalos while he killed 48 because “*Buffalo is an animal that breeds a lot*” (ID #6).

Table 3.1: List of hunted non-primate species, use of carcasses and hunting technique referred to during interviews. The GB common name and the English name are indicated, along with the scientific name and IUCN status: LC – Least concern, V – Vulnerable, NT – Near Threatened. The global population trend: D – decreasing, I – increasing, S – stable, U – unknown is also indicated (<http://www.iucnredlist.org/>)

GB name	English name	Scientific name	IUCN status	Use	Hunting Technique
Vaca de mato/ Bufalo	African buffalo	<i>Syncerus caffer</i>	LC, D	Meat consumption	
Cabra-mato	Maxwell duiker	<i>Cephalophus maxwelli</i>			
Gazela Pintado	Sitatunga, Marshbuck	<i>Tragelaphus spekii</i>			
Gazela de lala	Oribi	<i>Ourebia ourebi</i>	LC, D	Meat consumption/ Trade of horns for traditional medicine	Shotgun
Fritamba	Red-flanked Duiker	<i>Cephalophus rufilatus</i>			
Muntu	Yellow-backed duiker	<i>Cephalophus silvicultor</i>		V, I	
Elephant	Elephants	<i>Loxodonta africana</i>			
Pis-cabalo	Common Hippopotamus	<i>Hippopotamus amphibius</i>	V, D		
Lagarto	African Slender-snouted Crocodile (?)	<i>Crocodylus spp.</i>	?	Skin trade	Shotgun/ Nets
Porco-do-mato	Red river hog	<i>Potamochoerus porcus</i>	LC, D	Meat consumption	Shotgun
Javali	Common Warthog	<i>Phacochoerus africanus</i>	LC, S		
Sim-sim	Waterbuck	<i>Kobus ellipsiprymnus</i>	LC, D		
Boca branco	Roan Antelope	<i>Hippotragus equinus</i>			
Porco ispinho	Crested porcupine	<i>Hystrix cristata</i>	LC, U		
Pangolim	Long-tail pangolin	<i>Uromanis tetradactyla</i>	LC, D		
Pangolim	Giant pangolin	<i>Smutsia gigantea</i>	NT, D		
Farfana	Greater Cane Rat	<i>Thryonomys swinderianus</i>	LC, U		
Galinha de mato	Crested Guineafowl	<i>Guttera pucherani</i>	LC		
Manatim	Manatee	<i>Trichechus senegalensis</i>	V, U		
Onça	Leopard	<i>Panthera pardus</i>	NT, D	Meat consumption/ Skin trade	Shotgun

Table 3.2: List of hunted primate species, use of carcasses and hunting technique referred to during interviews. The GB common name and the English name are indicated, along with the scientific name and IUCN status: NT – Near threatened; E – Endangered; V – Vulnerable; LC – Least concern. It is also indicated the global population trend: D – decreasing, I – increasing, S – stable, U – unknown is also indicated (<http://www.iucnredlist.org/>)

GB name	English name	Scientific name	IUCN status	Use	Hunting Technique
Macaco Kom	Guinea baboon	<i>Papio hamadryas papio</i>	NT, U	Meat consumption/ Meat trade/ Pet trade	Shotgun/ Traps
Fatango	Western Red Colobus	<i>Procolobus badius</i>	E, D	Meat consumption/ Meat trade	Shotgun
Fidalgo	Western Black-and- White Colobus	<i>Colobus polykomos</i>	V, U		
Santcho di Tarrafe	Vervet monkey	<i>Cercopithecus aethiops sabaeus</i>	LC, S		
Santcho Fula	Patas monkey	<i>Erythrocebus patas</i>	LC, D		
Santcho Canculmena	Campbell's Monkey	<i>Cercopithecus campbelli</i>	LC, U		
Dari	Chimpanzee	<i>Pan troglodytes verus</i>	E, D	Pet trade	

Most of all non-primate species referred by the interviewees were hunted for meat consumption only. However, crocodiles and leopards were also hunted as a means to sell their skin, which was a very profitable activity in the past: [leopard skin] “*cost approximately 25,000 to 40,000 escudos [US\$ 0.87 - US\$ 1.48] a good price*” (ID #2); “*I killed four leopards. The first one, I sold it for 15,000 escudos. The same year, the second leopard I killed, I sold it for 17,000 escudos. (...) [Crocodile] I killed that too. You sell the skin depending on the length (...) (ID #6).* The price of the crocodile skin may have reached 50 - 500 CFA/cm (US\$ 0.92).

When the interviewees were questioned about animals that were harder to find or to see nowadays, the general opinion was that all large bodied size animals have decreased or disappeared (see Table 3.3). For example, buffalos considered abundant by the interviewees until the end of the war (1974), was frequently mentioned: *Animals that diminished? Buffalos disappeared. They are fewer now (ID #2); No, now there are no buffalo anymore. Previously, in the ancient time, local people hunted buffalo but now buffalo disappeared (ID #3); [Buffalos] are present but not a lot. They were more numerous in 1987 (ID #7).* The Roan Antelope is currently considered particularly rare and leopards are hard to detect: “*Roan Antelope and oribi, you don't see them anymore. (...) Waterbuck they exist but not that much*” (ID #3); *Roan Antelope is the one that decreased the most. There are still some buffalos and waterbucks (ID #6); Roan Antelope and leopard are the ones that decreased the most (ID #7).* “[Leopard] *just by chance you can see them*” (ID #2); “*Nowadays the leopards tend to hide well*” (ID #1). The interviewees justified their disappearance due to of over-exploration and/or avoidance of certain areas:

“*Buffalo, it is difficult to see, they just went to other places (...) [in Mampata] You can hunt (...) and even buffalo, because there is still a lot of buffalo there*” (ID #3); [Waterbuck and Roan Antelope] “*They disappeared maybe because they were all killed, or they ran away*” (ID #3); [Hippopotamus] “*Before (...) they were a lot but now just one or two (...) because the lakes got dried (...) they need a cooler place to stay*” (ID #7).

The typical behaviour described by the hunters was to hunt what they could find: “*We, hunters, we don't have feelings because we are hunters. The first thing that you see, you try to shoot it*” (ID #6); “*(...), I am not afraid to follow the lion. If I saw it I'd shoot it*” (ID #4); “*depends on what God allows you to kill*” (ID #6). The difficulty in finding one specific species may have lead to an increase of the hunting pressure towards another. For

example: [in the 1980's] (...) *you would walk 1 km to see a buffalo. Now, they all ran away because they were shot. So, I need to kill gazelle to eat* (ID #3); *“At that time, [in the 1980's] there were a lot of animals. I would come back with 5-6 gazelles. I would not shoot baboons”* (ID #7); *“Red colobus were more hunted because they were easily found (...) I would prefer baboon but the others were easier to detect”* (ID #7).

Table 3.3: List of species observed in the past by the interviewees and the respective hunter's classification regarding current abundance

Past	Nowadays
Leopard	Harder to detect
Yellow-backed duiker	No information
Pangolin	No information
Hiena	Abundant (Cufada)
Buffalo	Disappeared
Roan Antelope	Disappeared/ Decreasing
Waterbuck	Decreasing
Sitatunga, Marshbuck	Decreasing
Maxwell duiker	Still Abundant (Cantanhez); Decreasing (Cufada)
Red-flanked Duiker	Decreasing
Red river hog	Easy to detect
Oribi	Disappeared/ Decreasing
Elephants	Disappeared (Cantanhez); Present (Cufada)
Common Hippopotamus	Easy to detect (Cufada)
Crocodile	Harder to trap
Baboons	Harder to detect/decreased/ Still a lot
Patas monkey	Cufada: Few individuals/ Still possible to observe
Vervet monkey	Harder to see; Only next to mangroves
Western Black-and- White Colobus	Almost disappeared
Red Colobus	Almost disappeared
Campbell's Monkey	More common
Chimpanzee	Increased

The quantities obtained vary with the species body size, detection frequency, requirements of meat or money, consumer demand level and capability of transportation. Red colobus, for example, was mentioned as one of the easiest species to hunt: *Which monkeys are the easiest to hunt? Red colobus. Why? Because they don't go down [the tree] (...) they don't run away and you shoot at them and they fall down, and another one stays in the tree* (ID #7); [Red colobus] *“(...) they do not run away. You fire the weapon and they hide. They don't come down (...) and you can keep on chasing them (...) red colobus, they just stay in the tree above”* (ID #3), however the meat demand for this species does not seem to be large: [Red Colobus] (...) *“They are not bought a lot”* (ID #7).

The two hunters admitting to target baboons stated that they could hunt one or two baboons per day, four or five times a week. The amount of baboons hunted at one time seems to be constrained by their behaviour [*“No, it is not possible. If you shoot a baboon, the entire group will run away”* (ID #3)], perhaps by the current abundance [*“it could be 10, 20 up to 30, 40 [red colobus]. You can’t see that amount of baboons”* (ID #3)] and by its large body size [(...) *“you can’t take that much amount. Six or seven baboons are enough for your car (...). If I kill [baboon] I can transport up to five”* (ID #3)]. Hunting occurs usually during the night, in sleeping sites, which the hunters locate during the day. The males were described as being targeted first: *“Where they [baboons] sleep, we only look for the males. We can then try to get a female, but frequently we shoot the male first”* (ID #3).

In addition, baboons could be killed during crop-raiding conflicts or for the pet trade. However, chimpanzees seem to be more frequently targeted for pet traffic because baboons are considered *“stupid”* and *“restless”* pets that *“can break everything in the house”* (ID #3). It was not considered a straightforward task because it involved killing the lactating chimpanzee female without hurting the baby. In his own words: *“(…) it is not easy to catch a chimp baby; you have to kill his mother (...). You need to shoot (...) during the day. You sit down and wait. With mauser [rifle], you can kill the mother without hurting the baby but not with shotgun”* (ID #6). However, it seems to be highly profitable as a baby chimpanzee can be sold for US\$ 46 up to US\$ 56, compared with a baby baboon, which is sold for US\$ 2.69. In contrast with baboon pet trade, the costumers of chimpanzee were always referred to be foreigners (*white people*) and the interviewees considered the trade currently uncommon and more frequent in the past.

According with the interviewees, baboons were also hunted by militia. These practices were conducted during the night, at the baboons sleeping sites or during the day, where the baboons were detected: *“They hunted with Kalashnikov. (...) They would find out where [baboons] sleep and would stay, killing, until the sunrise. They (...) filled up the trucks and went (...) to gain money* (ID #2); *“The baboons did not run away from the cars (...) the militia would stop the car and start to hunt”* (ID #1). Nowadays, these practices are less frequent *“because they earn good money now”* (ID #2).

Baboons were classified as being abundant in the past but difficult to see nowadays: [baboon] *“The first monkey that existed here in GB (...) you could not compare with*

any other type. Baboons were a lot, abundant (...) there was more baboons than any other type of monkeys” (ID #2); [nowadays] *“today I need to walk two or three kilometres (...) or more (...) to find baboons. Before, you could stand here and we would see baboons on the road”* [Cufada Park road] (ID #3); *“there are not a lot [baboons] (...) before you would be obliged to drive slowly because the baboons crossed this road [Cufada Park road] frequently”* (ID #7). Difficulty in finding baboons was justified by either hunting-driven mortality: [Meat trade] *“that killed all monkeys in Guinea-Bissau. Since they started eating monkeys, they all died (...) [baboon] is decreasing everyday* (ID #2); *“Some of them [baboons] ran away but others were killed”* (ID #7); or avoidance of areas located nearby villages: *“Because of hunting and shotgun. If they saw someone, they think that they are going to die”* (ID #3); *“Baboons run away from people (...). If you don't have weapon, they are not scared, but if you have, baboons are scared”* (ID #7). Nevertheless, in areas where they persist, they are considered abundant: [baboon] *there are many (...) there are many baboons!* (ID #7); *Baboons? There are still a lot!* (ID #3).

Interviewees remembered that in the beginning of the meat trade (referred to be during the 1980's), a baboon carcass could be sold for approximately US\$ 0.44. A big male baboon would be worth more (US\$ 0.78 up to US\$ 2.53). Hunters indicated slightly higher prices for the current trade: a female baboon can be sold for US\$ 6.34 to US\$ 7.44, while big sized male baboons are worth US\$ 8 up to US\$ 11. The other species are sold for a lower price: a “normal” sized monkey for US\$ 1.27 up to US\$ 2.53, while a big one for US\$ 2.77 to US\$ 3.69. One interviewed said that in Bissau, a big monkey could reach higher values (US\$ 33) if sold directly to the bushmeat vendors or to the consumers. Therefore, although the carcasses are usually sold in nearby populated villages or along the main road, they would go to Bissau when they need cash urgently: *“(...) you need to resolve a problem (...) you can kill two or three monkeys to have money and (...) sell them but you will not have a lot of money. Therefore, it is better to go to Bissau to get more money. (...) However, it is not all the time, it is just when you have a problem* (ID #3). Campbell's monkey is currently the most consumed species (ID #3) and vervet monkey meat is greatly appreciated (ID #2), while baboons are considered to have an excessively strong flavour (ID #7).

The consumption of monkey meat is associated with alcohol drinking (a practice locally named *Abafatório*): *“(...) they would do Abafatório. (...) They prepare the monkey with onions and garlic, cook it well (...) then with beer (...) and other drinks, they serve a*

monkey meat dish. While people are eating, they are buying drinks (ID #2). The use of monkey's meat in the village's *abafatório* practices was justified by the lower cost: *"But they [monkey meat consumers] could not eat a chicken instead?" "A bullet is 350 FCA [US\$ 0.63], a chicken is 1,000 FCA [US\$ 1.85] so it is better to buy a bullet and go hunting"* (ID #5). The hunters can also sell the carcasses directly to the restaurants owners: *"Well, if you are a hunter, you know that people selling wine need meat (...) you can negotiate"* (ID #3). This dish would cost between US\$ 1.58 and US\$ 5.54.

Nowadays, hunting practices targeting primates is a widespread activity: *"Any village (...) has hunters"* (ID #2) and *"All villages have those [baboon] hunters"* (ID #3). The money obtained through this activity seems to be important in their daily lives: *"I killed a female baboon in Cangode. In that day, I didn't have money"* (ID #5) and the bushmeat trade seems to represent wealth for the stakeholders: *"Lots of money! They earn lots of money in that"* (ID #2). However, the generalization of hunting activities seem to be associated to more immature and younger hunters, as a mean to obtain cash quickly. For example: *"We don't have normal hunters here. (...) I am a big hunter (...). But, there are not hunters here, just shooters. You hunt in your house and you shoot in the forest"* (ID #4). *"They contacted (...) younger guys, infants, younger hunters (...) to kill the monkeys and sell them at a symbolic price"* (ID #2). *"A lot of hunters there. Young people are almost all hunters there"* (ID #3). Most interestingly, the youngest hunter justified this behaviour because of jobs shortage: *"As a young person, every morning you wake up but you don't have a job. But, you need clothes, and if you are a smoker, you need cigarettes. If you don't hunt you will not have anything. That's why all young people hunt"* (ID #3).

In most cases, concern was demonstrated about hunting within the protected areas. The control against hunting practices in Cantanhez started in 1994: *"(...) they raised awareness in the radio [community radio] and told all the villages to stop hunting. They told that we are respected men and we have families. It would be shameful to mention our names in the radio if we did something wrong"*(ID #4). When someone is acting illegally, they must apologize for their actions (sometimes to the *régulo*, the King), which bring *shame* to the perpetrator and his family. This can influence their cost/benefit analysis: *"The "sacred" forest [protected area] is for all of us. I will not go in there to hunt. If someone sees me hunting there, he would tell me to stop (...). If you finally do it, and someone sees you, the [the law] will be applied. Instead of apologizing, it is better not to go hunting (...)"* (ID #4). As told by the interviewees, in Cufada Park the guards will keep the gun and the hunters will be fined in

25,000 FCA (US\$ 40). If the hunters are not resident in the Park, the fine will be higher (50,000 FCA, US\$ 79) (ID #3).

The Cufada interviewees stated that nowadays, hunting primates, as a means to supply the bushmeat trade in Bissau, is infrequent within the park: *“Just local people hunt here in the park. Because, if you come from outside and we see you, we will send you name to Buba [Park headquarters] (...) we’ll not accept because we don’t let them finish with all the animals”* (ID #3); [hunters in the park?] *To consume yes, but now there are not* [hunters from Bissau]. *There is a lot of control in Quebo and Bambadinca (...) [monkeys that arrive in Bissau?] But they don't come from here”* (ID #7).

Although the interviewees recognize the benefits of a protected area [*“I don’t know anything about the school of white people. If they say that the park is good, I accept”* (ID #4)], they complained about the control exerted towards their activities: *“Because people from Cantanhez are not happy. They cannot do anymore what they did in the past because of the park rules”* (ID #4). The lack of alternative activities seems to causing the dissatisfaction towards the park management: *“How can I eat without cultivating and hunting?”* (ID #6); *“They can’t make me stop working in the field, fishing, or hunting if they don’t give me anything in return. If they don’t want me to die, they should give me something to eat. All that I do it is for my family”* (ID #4); *“If they [Park management] accepted to collaborate with the people living in the park, the park would be ok. (...) We could build the park all together (...) Anything you do, is wrong”* (ID #3).

3.5 – Discussion

We found 150 primates at Bissau bushmeat markets across the 19 days of the study. The estimated number of primates being traded (2008 specimens/dry season) seems to confirm the generalized notion of severe hunting practices towards primates in GB (Casanova and Sousa, 2007; Gippoliti and Dell’Omo, 2003). Although already high, these figures are probably underestimated. The animals consumed at the villages, which can reach hundreds of animals annually (e.g. Fitzgibbon *et al.*, 1995), and the ones sold alongside the road were not counted at the bushmeat markets (Chapman and Peres, 2001).

Primate meat consumption in GB was associated with alcohol consumption, a practice locally called *Abafatório*. The meal, costing around US\$ 2.63 in Bissau, is

considered expensive in an urban GB context, which suggest preference towards primate meat. Urban consumers might regard bushmeat as an extravagant diet item (Pailler, 2005; Wilkie *et al.*, 2005), relate it to rural ancestry and past dietary traditions (Mbete *et al.*, 2011) or considerer it to be more healthy (Easta *et al.*, 2005; van Vliet and Mbazzab, 2011).

The results from the interviews suggest a different profile for rural consumers. Although consumption was associated with alcohol, the preference for primates was related to its lower cost when compared with domestic meat. The supply of domestic meat is insufficient in GB. When compared with the surrounding countries, Guinea-Bissau residents consume the lowest amount of domestic meat (e.g. pigs, cows, chickens, goats) due to a limited production, conservation and distribution (DGP, 2002). Frequently, in rural areas in southern GB, domestic meat is produced by a few ethnic groups and consumed only on special occasions (e.g. funerals, births and weddings) (Costa, 2010). Locals need to buy meat from their neighbours or exchange it for other goods. In addition, although fisheries are an important economic activity in GB (World Bank, 2004), its supply is brief and irregular (Costa, 2010). Therefore, bushmeat consumption in rural areas might be accentuated by variation in agricultural production (de Merode *et al.*, 2004) and fisheries (Brashares *et al.*, 2004; Wilkie *et al.*, 2005). Whether, social or culturally related causes, palatability or will to diversify dietary items (Bowen-Jones, 1998; Fa *et al.*, 2003; Schenck *et al.*, 2006; van Vliet, 2011; Willcox and Nambu, 2007), may also play a role.

At urban markets, the peak of the trade (17 specimens/day) coincided with popular festivities (such as the Carnival or the Easter), which is usually associated with alcohol consumption. The traders justified the major decline in supply during the last period of the study as a decrease in hunting practices during the rainy season, which the interviewees refuted. Alternatively, the lower hunting rates may be related with limited accessibility to isolated areas or associated with an increase of the transportation costs to urban centres (Bennett *et al.*, 2002; Fa and Brown, 2009; Wilkie *et al.*, 2000). Guinea-Bissau lost most of its infrastructure during the last civil war (1998) and the roads to the southern areas frequently become impassable during the rainy season (Ferreira da Silva, personal observation).

The molecular identification protocol allowed a more accurate determination of the species traded, as found by Olayemi *et al.* (2011). The use of COI as a molecular marker was efficient in our case, as the intraspecific variation was low. The analysis produced a phylogeny in which samples clustered together with the voucher sequence with strong bootstrap support. The fact that divergence between species clade is substantially higher than within each clade, and that the six monophyletic groups corresponded to the six identified species, gives strong support that this method correctly assigned all samples to their true species (Waugh 2007). Nevertheless, *C. campbelli* was only identified using the 12S rRNA fragment because the databases lacked appropriate vouchers for this species, which constitutes one drawback of DNA barcoding (Frézal and Leblois, 2008).

Ignoring possible misidentifications in cases of smoked carcasses can lead to inadequate conservation policies and efforts (Teletchea *et al.*, 2005). In our study, the molecular identification revealed the trade of *C. sabaesus*, which was unspecified before. After correction by molecular identification, *C. campbelli* still remained one of the most traded species, but the relative proportion in the trade decreased by 60% of the proportion estimated using morphology only and *C. sabaesus* became the most traded species. These findings emphasise the importance of the molecular identification protocols in studies assessing the species-specific relative proportions (Baker, 2008).

From the vendors' perspective, the price at the markets depended on body mass. Similarity between *C. campbelli*, *C. sabaesus* and *P. badius* body weights seem to explain these species high morphological error rates and the traders' frequent misidentifications. Baboons, on the other hand, having the highest and more distinctive weight of all primate species traded (see Fig 3.9) presented lower error rates. Interestingly, in one case of misidentification, baboons were confused with *E. patas*, a species overlapping with *P. h. papio* female weights (Fig. 3.9).

Alternatively, variation of common names across vendors could also explain misidentification between *C. campbelli* and *C. sabaesus*. *C. sabaesus* was named by some interviewees in Cufada as *Mona monkey*, which is the name for *C. campbelli* in Cantanhez region. In Bissau, as the price is established based on size, the bushmeat market vendors may refer to both species interchangeably as *Mona monkeys*.

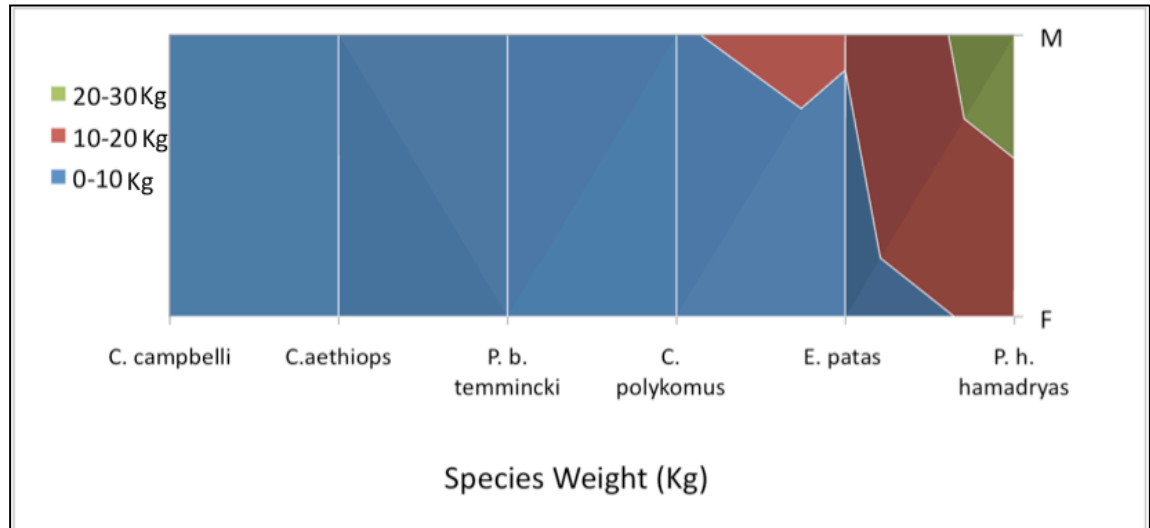


Figure 3.9: Area chart representing the weights (kg) of primate species being traded at the Bissau markets. Colours represent classes of weights (green: between 20 to 30 Kg; red: between 10 to 20 Kg; blue: between 0 to 10 Kg) for Males (M) and Females (F). [Note that the weights for the species are from Fleagle (1988) and might vary from those of the GB populations].

At this point, we can only speculate why *C. sabaesus* and *C. campbelli* are the most traded species. According with the hunters, the species-specific harvesting rates varied with the species body mass, frequency of encounters, cash requirements and consumers demand. Hunters suggested that *C. campbelli* is relatively easy to find and *C. sabaesus* meat is greatly appreciated, which could justify their high trade rates. Moreover, hunting activities towards primates were associated with a faster resolution of unexpected financial problems and a means to obtain fast cash, similarly to what was found in Gabon (Coad *et al.*, 2010) and already suggested by Casanova and Sousa (2007) for GB. In such cases, the hunters might not spend a great amount of time searching for a particular species and will target the more available species. This can be the case for both *C. campbelli* and *C. sabaesus*, as these species can persist in disturbed habitats (Kingdon and Gippoliti, 2008; Oates *et al.*, 2008) and are thought to have a widespread distribution and high abundance in GB (Gippoliti and Dell’Omo, 2003).

Nevertheless, both hunters and vendors would earn twice as much with male baboon meat. As baboons were only the third most traded species at the markets, this finding suggests: 1) lower preference of its meat by consumers or 2) difficulty in finding that species, as previously stated by the hunters interviewed by Cá (2008). Even though one interviewee claimed that baboons have an excessively strong flavour, it remains possible that baboons are currently harder to find within Guinea-Bissau.

In Guinea-Bissau, Guinea baboons were shot, trapped or hunted with dogs, for meat consumption (in the villages) or for meat trade (in bigger cities). Additional mortality can also arise from crop-raiding conflicts (Costa, 2010). Baboons were also hunted by militia as a replacement of their salaries, already described by Casanova and Sousa (2007). This relationship between the armed forces and wildlife depletion has been observed in other locations (McNeely, 2003) and might have also contributed to decrease in the species' abundance in the past. The current hunting rate suggested by the interviewees (of one or two baboons per day, four or five times a week) and the estimated numbers of baboons being traded at the markets (387 specimens/dry season) suggest current high levels of hunting-driven mortality for this species and points to a population decline (Casanova and Sousa, 2007; Gippoliti and Dell'Omo, 2003).

However, although the hunters stated they were *not seeing baboons* as frequently as before they still perceived baboons as a relative abundant species. One possible explanation for this apparent contradiction is a behavioural change to avoid detection by hunters (e.g. Croes *et al.*, 2006; Cowlshaw and Dunbar, 2000), in which areas close to villages and human infrastructures are currently being avoided.

Two of the interviewees referred to target male baboons first. This behaviour can be related with profit maximisation. Male baboons represent higher economic return for the investment in ammunitions and if the common behaviour of the baboon group is to immediately disperse (in contrast to other species), when possible, the hunters will tend to shoot first at male baboons. Nevertheless, it is quite common to observe baboons being kept as pets throughout GB (Hockings and Sousa, 2011) and this suggests high mortality levels in females. However, baboons do not seem to be targeted as frequently for pet trade as chimpanzees because: i) baboons are not considered "good" pets and ii) the trade does not seem to be as profitable. Therefore, the "baboon pet trade" is probably a result of meat-driven hunting practices. The hunters can shoot at lactating baboon females (with the purpose of obtaining meat) and keep or sell the dependent juveniles.

The typical behaviour of the hunters has been to target big animals (e.g. ungulates) and they were not commercial hunters targeting primate species. However, they suggested that the low abundance of a previously targeted species led to hunting practices for other more abundant species. If this was the general tendency among GB hunters, the population decline of the GB ungulates, elephants and felids (Brugiere *et al.*,

2005; Brugiére *et al.*, 2006) might have contributed to increased hunting pressure towards primates for subsistence purposes. Within this context, hunting pressure probably increased first towards the Guinea baboon, as this species is the biggest in body mass of all primate species being consumed or traded.

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Chapter 4 Genetic diversity and structure of a hunted baboon population (*Papio hamadryas papio*) in Guinea-Bissau, West Africa

Gatu fartu ka ta montia

(A well-fed cat does not hunt –

Popular saying in Guinea-Bissau suggesting that hunger leads to hunting)

Genetic diversity and structure of a hunted baboon population (*Papio hamadryas papio*) in Guinea-Bissau, West Africa

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4.1 - Abstract

In Guinea-Bissau Guinea baboons are heavily hunted for meat consumption and are thought to be undergoing a significant range contraction within the country. Hunting pressure can lead to important changes at the population level, not fully understood in primate species. In this work, we used two different genetic markers (fourteen microsatellite loci and a fragment of the mitochondrial control region) and a non-invasive sampling strategy in southern Guinea-Bissau to assess if the mortality caused by hunting practices constitutes a barrier to dispersal. After evaluating genetic diversity and population structure, we assessed if genetic discontinuities in the data were concordant with the location of human pressure by using a landscape genetics approach. In addition, we used molecular markers to assign bushmeat tissue samples to their most probable population of origin. We found that, despite high hunting-driven mortality, the genetic diversity was not significantly reduced when compared with other Guinea baboon populations. Evidence of historically female-biased dispersal and more recent contact between localities was found, along with admixture between sampling regions. Geographic distances had a weak effect on population divergence and the underlying social structure could only partially explain the patterns revealed. Evidence was found of a contact zone where gene flow seems to be unidirectional and where admixed individuals are in higher proportion. These genetic discontinuities are not related with natural or anthropogenic barriers to gene flow but are concordant for both genetic markers. Our results suggest that hunting pressure has caused recent contact between genetically differentiated individuals, which now co-exist in the same social unit.

Keywords: Hunting Pressure, Population Structure, Genetic Diversity, Guinea Baboons, Landscape genetics

4.2 - Introduction

The impact of hunting on wildlife populations can include changes in the demographic and hence genetic structure, observations that are usually less obvious than population declines and even direct extinction caused by over-harvesting (Harris *et al.* 2002; Coltman, 2008; Allendorf and Hard, 2009). Hunting pressure can alter population structure by decreasing the number of migrants between breeding groups (Allendorf *et al.*, 2008). This effect can, in principle be especially pronounced if the dispersing sex is targeted by hunters (Ellsworth *et al.*, 1994), if a reduction in the number or density of subpopulations limits dispersal (Allendorf *et al.*, 2008) or if human settlements constitute important barriers to gene flow (e.g. Liu *et al.*, 2008; Murtskhvaladze *et al.*, 2010). As a consequence, groups may lose genetic variation through drift, become more inbred and may lose fitness as a consequence (Allendorf *et al.*, 2008). Alternatively, gene flow can be increased (Harris *et al.*, 2002, Allendorf *et al.*, 2008): as a result of a defensive behavioural response such as the forming of larger groups (Nyakaana *et al.*, 2001), as a result of lower population density and consequent higher dispersal distances (Allendorf *et al.*, 2008) or by demographic “sinks” created by spatially intense and varied hunting practices (Harris *et al.* 2002, for example Delibes *et al.*, 2001; Jedrzejewski *et al.*, 2005; Robinson *et al.*, 2008). To create demographic sinks, individuals may be removed by hunting and gene flow from less exploited areas may become proportionately more significant in altering allele frequencies (Harris *et al.*, 2002). Potential consequences of demographic “sinks” are i) a decrease in the effective population size (Coltman, 2008) and ii) contact zones, where allopatrically differentiated sub-populations exchange genes and genetic “swamping” and loss of local adaptations might occur (Allendorf *et al.*, 2008). Thus, apparently restricted harvesting practices can impact the genetic diversity of the population at a broader scale than initially considered.

Hunting practices are expected to structure primate populations (Allendorf *et al.*, 2008), as most non-human primate species are a target for either commercial or subsistence hunting (Chapman *et al.*, 2006; Chapman and Peres, 2001; Di Fiore, 2004; Mittermeier *et al.*, 1999; Mittermeier, 1987). Ecological, behavioural and demographic hunting-driven changes have been identified in primate species (Cowlshaw and Dunbar, 2000) however the extent to which the population dynamics are affected in African bushmeat species is still not well understood (Fa and Brown, 2009).

Baboons (*Papio hamadryas spp.*) are often hunted when they live alongside human settlements and when they represent significant crop-raiding populations (Altmann and Muruthi, 1988; Biquand *et al.*, 1992b; Brugiere and Magassouba, 2009; Fitzgibbon *et al.*, 1995; Kansky, 2002; Nowak, 1999; Starin, 1989; Wolfheim, 1983). Meat consumption has been described so far mainly as a by-product of pest control activities, either at the local or regional scale (Brugiere and Magassouba, 2009; Fitzgibbon *et al.*, 1995) and baboons are frequently referred to as a traded species in bushmeat markets (e.g. Bowen-Jones and Pendry, 1999; Brugiere and Magassouba, 2009). Meat-driven or pest control hunting activities have also lead to the establishment of a significant pet-trade, on a local or even international scale (Mittermeier, 1987; Starin, 1989). IUCN does not regard baboons as a priority for global primate conservation (since they are included in the category of Lower Risk category and in CITES annex II, IUCN, 2007). Nevertheless, a hand-full of studies suggests that the interference with human activities results in an acute reduction in numbers and range contraction in populations (Biquand *et al.*, 1992; Fitzgibbon *et al.*, 1995; Galat *et al.*, 1999-2000; Kansky, 2002; Nowak, 1999; Wolfheim, 1983).

In Guinea-Bissau, a country in West Africa, baboons (*Papio hamadryas papio*) are mainly hunted for meat consumption, either at the local level (in the villages, as a substitute of domestic meat, Costa, 2010) or at the regional level (sold at the bushmeat markets or consumed in restaurants in the capital city, Bissau, Cá 2008; Casanova and Sousa, 2007). Crop-raiding conflicts (Costa, 2010) and hunting episodes performed by the military, where large quantities of baboons were killed (Casanova and Sousa, 2007), have contributed to the species' population dynamics. Furthermore, baboon skins are used for traditional medicine purposes (Ferreira da Silva *et al.*, 2009; Sá *et al.*, 2012) and, most likely, because of meat-driven hunting practices, it is common to observe young individuals being kept as pets throughout the country (Casanova and Sousa, 2007; Hockings and Sousa, 2011). This apparently high hunting pressure that may have started in the 1980's (Casanova and Sousa, 2007) or even earlier (1970's Cá, 2008, 1963-1974 Ferreira da Silva, unpublished data), along with increased habitat loss, is thought to be causing a major decline in the population (Gippoliti and Dell'Omo, 2003; Casanova and Sousa, 2007; Cá, 2008; Costa, 2010). Baboons are believed to still be relatively common in the southern area of the country (Cantanhez and Boé region) (Gippoliti and Dell'Omo, 2003) but residents of those areas also claim to see baboons less frequently (Costa, 2010) and hunters state that baboons are becoming rare and more difficult to find (Cá, 2008).

In Guinea-Bissau the bushmeat trade occurs along the main (and only) tarred road that connects the southern part of the country to the capital city (Cá, 2008; Casanova and Sousa, 2007). An intermediate agent in the trade (locally called *bideiras*) orders the bushmeat directly from the hunter providing him with cartridges or payment in advance (Cá, 2008). The *bideiras* transport the bushmeat to Bissau, where the carcasses are sold in meat markets or to specialised restaurants (Cá, 2008; Chapter 3). At these restaurants, primate meat is commonly consumed with alcoholic beverages as an expensive delicacy (Starin, 2010).

Some evidence suggests that baboons, of all primate species sold in the Guinea-Bissau's bushmeat trade, might represent the preferred species targeted by hunters. The trade of baboon meat appears to be highly profitable. Male baboons are traded at a price 60% higher than the other primate species at urban bushmeat markets (Chapter 3), which is probably related to its bigger body mass (Macdonald *et al.*, 2011). Nevertheless, baboons are only the third most traded species of the six species found at Bissau bushmeat markets suggesting either a lower consumer preference towards baboon meat or a recent increased difficulty in finding them (Chapter 3).

The aim of this research was to investigate if the human settlements (used here as a proxy of hunting pressure) could constitute a barrier to dispersal in this primate species. We conducted non-invasive genetic sampling in two protected areas (Cantanhez Woodlands National Park and Cufada Lagoon Natural Park) and in one remote area (Boé region) with a lower density of human populations. The sampling areas are separated by a considerable number of human settlements and by the road that connects the south of the country to the capital. As baboons exist in low densities or have recently disappeared at these locations, we expected to find a pattern of concomitant genetic differentiation between sampling regions. We used two genetic markers, fourteen microsatellite loci and 393 base pairs (bp) fragment of mitochondrial DNA (mtDNA) of the control region to: i) describe the genetic diversity present in the southern part of the country; ii) characterise population structure using individual-based Bayesian methods and iii) assess if the main genetic discontinuities are concordant with the location of human pressure. Additionally, we also aimed to infer the geographic location of heavily hunted areas within the country by assigning bushmeat tissue samples collected at the markets in Bissau, to their source population.

4.3 – Methods

4.3.1 - Samples Collection

Non-invasive faecal sampling collection in Guinea-Bissau (GB) took place in the southern and eastern part of the country where Guinea baboons are reported to exist (Gippoliti and Dell’Omo, 2003) in three different regions: Cantanhez Woodlands National Park (*Parque Nacional das Matas de Cantanhez*), Cufada Lagoons Natural Park (*Parque Natural das Lagoas da Cufada*) and Boé Region. Cantanhez and Cufada are two protected areas 40 Km apart from each other, and are located in the southwestern part of the country, separated by roughly 100 Km from Boé Region that is located in the southeast (see Fig 4.1 and Table 4.1). Local villagers and park guards and guides helped to localise social units (mostly foraging parties, with the exception of one sleeping site, at Bubatchingue site, Table 4.1). Social groups are defined as groups of samples collected in close proximity (not distanced more than 700 meters). Samples were collected fresh, a few minutes after detection of the groups. Five ml of the faecal material, scraped from the exterior surface of the bolus, was preserved using a two-step method described in Roeder *et al.*, (2004). This method comprises a preliminary 24h treatment using 99% ANALAR ethanol, followed by preservation in silica gel (Type III, S-7625, indicating for desiccation, Sigma-Aldrich® Company Ltd, Dorset, UK). Fourteen tissue samples, collected in the bushmeat market in Bissau (Chapter 3), were preserved in 99% ANALAR ethanol. Hair samples were obtained from captive individuals. All samples were maintained at room temperature until DNA extraction.

4.3.2 - Laboratory methods

Total genomic DNA from faecal samples was extracted using the QIAamp®DNA Stool Mini Kit (QIAGEN ®) with the following modifications from the manufacturer’s protocol: scraping the outer part of the sample with a blade, immersing the sample in ASL Buffer overnight, increasing action period for the InhibitEX Tablet to 10 minutes and Proteinase K digestion 30 minutes. DNA extracts, eluted in 200ul AE Buffer, were stored at -20°C. Negative controls were subjected to all extraction procedures and included in subsequent PCRs. Whole genomic DNA from bushmeat tissue samples was extracted using the DNeasy Blood and Tissue kits (Qiagen ©) following the manufacturer’s instructions with few modifications (Chapter 3).

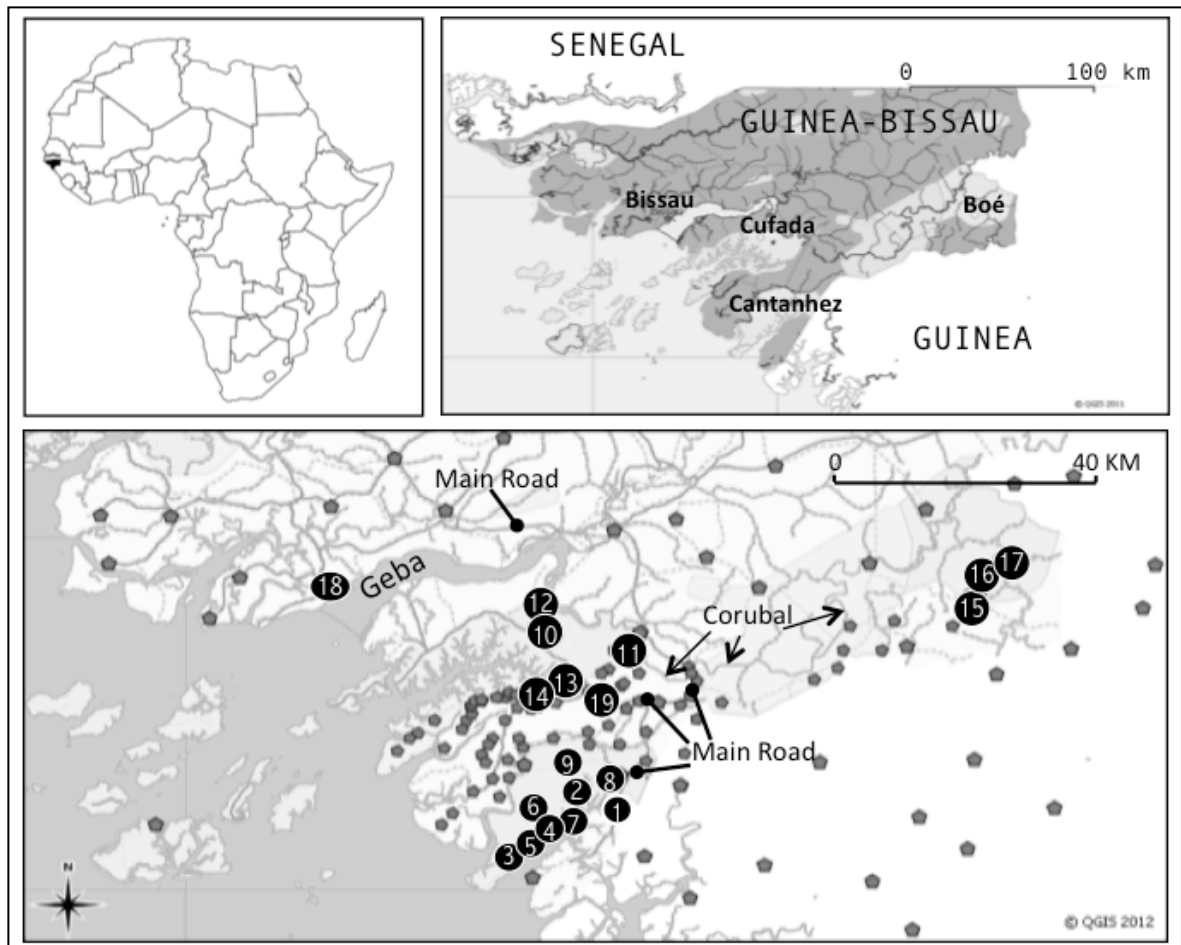


Figure 4.1: Map of study site location within both West Africa and Guinea-Bissau and map of sampling regions. The main anthropogenic and natural features are indicated [main villages and road are indicated by pentagons and by a continuous line, respectively; the main rivers - Geba and Corubal - are represented (Corubal is indicated by arrows)]. Black dots represent social units according to its location and numbered as in table 4.1.

4.3.2.1 - *Microsatellite Loci*

Fourteen autosomal microsatellite loci were amplified in four multiplex and one singleplex PCR reactions. Markers were amplified in 10uL volume using the QIAGEN Multiplex PCR Kit ®, using 2uL of DNA extract. The final reaction concentrations consisted of 1x QIAGEN Multiplex PCR Master Mix ®, 0.2uM of each multiplex primer mixture and 0.75uM of BSA (see Table 4.2 for multiplex PCR conditions and concentrations of primers in the final reaction). PCR cycling conditions started with a HotStarTaq DNA Polymerase activation step (15 min at 95°C), followed by 40 cycles of denaturation at 94°C for 30 sec, annealing step for 40 sec at between 50°C to 59°C (Table 1) and extension at 72°C for 60 sec. The PCR ended with a final extension of 30 min at 72°C.

PCRs were performed in an AB Applied BiosystemsTM (California, USA) Veriti 96 Well Thermal Cycler. In order to avoid cross-contamination between samples and external DNA contaminations, all material used were UV light irradiated and PCRs were assembled in a Microflow OMNI PCR workstation (Bioquell UK Ltd, Hampshire, UK) using sterile filter tips in all steps. Samples were analysed using Macrogen Korea's Genescan service and run on an ABI3730XL capillary analyser. Multiplex 1, 3, 4 and 5 were analysed using a 16 GeneScanTM -500 LIZ ® size-standard and multiplex 2 was analysed using 16 GeneScanTM -400 HD ® size-standard.

Table 4.1: The table indicates the number of samples used in the analyses using the two genetic markers (microsatellite loci and mtDNA D-loop) and name and location of sampled social units.

Sampling Region	Map	Social unit	Microsatellite loci	mtDNA
Cantanhez	1	Porto Gadamael	13	18
	2	Amindara Catobo	7	8
	3	Cabedu	9	10
	4	Catomboi	10	11
	5	Canamina	10	11
	6	Caiquene	4	4
	7	Cambeque	6	10
	8	Quebo-Sutuba	4	4
	9	Botchê Cule	10	10
		Total Cantanhez	(73)	(86)
Cufada	10	Bubatchingue	21	20
	11	Bakar Contê	11	12
	12	Guebombol	5	5
	13	Sr.Soures1	7	9
	14	Sr.Soures2	10	10
		Total Cufada	(54)	(56)
Boé	15	Boé Beli	6	6
	16	Boé Aicum	11	11
	17	Boé Montanha	5	6
		Total Boé	(22)	(23)
Bissau	18	Tissue samples	14	4
		Hair Samples	0	4
Buba	19	Hair Samples	0	2

The genotyping followed a modified “multi-tubes” approach (Taberlet *et al.*, 1996). Allelic dropout (ADO) and false allele rate (FA) were estimated on an initial dataset of 80 samples using a maximum likelihood approach (Johnson and Haydon, 2007a), implemented in Pedant version 1.0 (Johnson and Haydon, 2007b). Based on these results, we estimated the number of repetitions across loci required to obtain 95% confidence in the genotypes using GEMINI version 1.4.1 (Valière *et al.*, 2002). Four amplifications per locus per sample

were performed and the consensus threshold estimated by GEMINI was used to set the genotyping rules for the samples. We quantified the reliability of genotypes using the “quality index” (QI) (Miquel *et al.*, 2006) and included samples with a QI above 0.55 in the final dataset (Miquel *et al.*, 2006). Amplification success, ADO and FA were then estimated for samples included in the final dataset (Table 1). Amplification success per locus was calculated based on all amplifications attempted. ADO and FA for each locus were estimated according to Broquet and Petit (2004). Tests to detect repeat-sampled individuals were performed using the Excel-based microsatellite toolkit (Park, 2001), allowing for one mismatch or two for samples distinguished by only one locus with a homozygote genotype. The probability of identity (Waits *et al.*, 2001) was computed using GIMLET version 1.3.2 (Valière, 2002).

Table 4.2: Details of microsatellite PCRs performed. Name of loci included in each multiplex PCR plus annealing temperature, GenBank accession for each locus and final concentration of each primer in the reaction, Allelic range (in bp), Amplification Success, Allelic Dropout Rate (ADO) and False allele rate (FA).

PCRs	Locus	GenBank Code	Final concentrations (uM)	Allelic range (bp)	Amplification Success (%)	ADO (%)	FA (%)
M1 (57°C)	D13S765	G09003	0.2	200-212	77	16	3
	D12S375	G08936	0.1	164-184	79	13	3
	D3S1766	G08269	0.1	192-208	85	14	3
	D7S503	G18277	0.6	142-156	82	15	2
M2 (55°C)	D2S1326	G08136	0.3	192-208	67	32	1
	D14S306	G09055	0.2	161-181	92	11	2
	D1S533	G07788	0.4	187-203	59	31	4
M3 (59°C)	D8S1106	G09378	0.1	149-161	94	11	2
	D6S501	G08551	0.5	171-187	94	20	5
	D10S611	G08794	0.1	129-137	80	28	1
M4 (57°C)	D5S1457	G08431	0.1	125-137	75	22	4
	D7S2204	G08635	0.4	230-250	86	15	2
	D3S1768	G08287	0.1	193-212	90	16	2
SINGLEPLEX (58°C)	D4S243	M87736	0.2	152-172	77	7	1

Locus-specific deficiency in heterozygotes due to null alleles, stutter band-related scoring errors and large-allele dropout was tested using Micro-Checker version 2.2.3 (Oosterhout *et al.*, 2006), with a 95% confidence interval.

4.3.2.2 - *Mitochondrial DNA*

A fragment of approximately 490bp of the mtDNA control region (hypervariable region I) was amplified and sequenced, using the primers designed by Hapke *et al.*, (2001)

(L15437: CTGGCGTTCTAACTTAACT and H15849: GTAGTATTACCCGAGCGG). The fragment was amplified in 22uL using the QIAGEN Multiplex PCR Kit ®, containing 1.5uL of DNA extract. The final reaction concentrations consisted of 1x QIAGEN Multiplex PCR Master Mix ® and 0.2uM of each primer and 0.75uM BSA. The PCR started with a HotStarTaq DNA Polymerase activation step (15 min at 95°C), followed by 40 cycles of denaturation at 94°C for 30 sec, annealing temperature at 59°C for 90 sec and extension at 72°C for 60 sec. The PCR ended with a final extension of 30 min at 60°C. PCRs were performed in an AB Applied Biosystems™ (California, USA) Veriti 96 Well Thermal Cycler. After an electrophoretic assay, PCR products were purified with 10 units of Exonuclease I and 5 units of Antarctic Phosphatase (New England Biolabs Inc., Ipswich, UK). Conditions for PCR product purification started with a 30 minutes step at 37°, followed by 20 min at 80°C and 5 min at 12°C. Samples were then sequenced bidirectionally by Macrogen Europe's EZ-seq direct service, using an ABI3730XL capillary analyzer.

Sequences were manually checked for accuracy using Sequencher v4.9 (Gene Codes Corporation, USA), a consensus was created using both the forward and reverse sequence of each sample and all polymorphic positions were checked by eye for each sample chromatogram. Sequences were then exported into BioEdit version 7.0.9 (Hall, 1999), aligned and trimmed to 393bp (corresponding to the length of the shortest sequence).

4.3.3 - Statistical analysis

4.3.3.1 - Genetic Diversity

4.3.3.1.1 - *Microsatellite loci*

Genetic diversity levels were preliminarily assessed using expected (H_e) and observed heterozygosity (H_o), unbiased expected heterozygosity (UHe), mean number of alleles per locus and inbreeding coefficient (Fis) computed using GENETIX version 4.05 (Belkhir *et al.*, 1996-2004). Allelic richness, compensating for unequal sample sizes, was computed using FSTAT version 2.9.3.2. (Goudet, 2002). An exact test for Hardy-Weinberg equilibrium at each locus, linkage disequilibrium between all pairs of loci and pairwise Fst were computed between sampling regions using Arlequin version 3.11 (Excoffier *et al.*, 2005).

4.3.3.1.2 - *Mitochondrial DNA*

Mitochondrial DNA summary diversity statistics were computed using DNAsp v5 (Librado and Rozas 2009). Number of haplotypes, Haplotype Diversity, (Hd; Nei 1987), Nucleotide Diversity (π ; Nei 1987) and respective standard deviation (SD) were estimated for sampling regions. Pairwise *Fst* between sampling locations, based on haplotype frequencies and pairwise differences (Nei and Li, 1979) and its significance (assessed using 10,000 permutations) was computed using Arlequin v3.11. To explore the relationship between haplotypes, a median joining network was constructed using NETWORK version 4.6 (Bandelt *et al.*, 1999). An initial network was constructed with equal weights among all polymorphic sites but was not well resolved due to two rapidly evolving characters (character 273 and 248). Based on this result, as recommended by Bandelt *et al.* (1999), these frequently changing nucleotide positions were down weighted (Bandelt *et al.*, 1999).

4.3.3.2 - Identification of subpopulation units

4.3.3.2.1 - *Microsatellite loci*

We performed a Mantel test and a spatial autocorrelation analysis to assess the correlation between genetic distance and geographic distance using GenAlEx v6.4.1. (Peakall and Smouse, 2006). In GenAlEx, the Mantel correlation coefficient (R_{xy}) varies between -1 and 1 and significant differences from $R_x = 0$ (no correlation between genetic and geographic distances) are assessed by random permutation. We used 9999 permutations. The spatial autocorrelation coefficient (r) - a measure of the genetic similarity between pairs of individuals in a specified distance class - was calculated for 10 distance classes (16.5 to 165 Km). This statistic is closely related to Moran's-I and varies between -1 and 1 (indicative of genetic dissimilarity and genetic similarity, respectively) (Peakall and Smouse, 2006). Significance was assessed using the bounds of a 95% confidence interval, which was obtained by permutation (9999 permutations were used). The results were summarised by a correlogram. An analysis of molecular variance AMOVA (Excoffier *et al.*, 1992) was performed at three hierarchical levels (within and among social units, within and among each sampling location and overall) using Arlequin v3.11 (see Fig. 4.1 and table 4.1 for definition of social units used in the analyses).

We used three different individual-based Bayesian software packages to identify population structure: STRUCTURE version 2.1 (Pritchard *et al.*, 2000), BAPS v5.2

(Corander *et al.*, 2006; Corander *et al.*, 2008a) and GENELAND version 3.3.0 (Guillot *et al.*, 2005b). Clustering of individuals using STRUCTURE was carried out using the “admixture model” (with parameters set to default), assuming either independent allele frequencies (e.g. drawn from a distribution) or correlated allele frequencies (e.g. similar frequencies due to migration or shared ancestry). We inferred clusters between 1 and 10. Each run was preceded by a burn-in of 100,000 steps followed by four MCMC runs of 1,000,000 iterations. To test for convergence of the posterior distribution, the analysis was repeated three times. To determine the optimal number of clusters (K value), we estimated the posterior probability of K (Pritchard *et al.*, 2000), the highest estimated log-likelihood [$\ln P(X/K)$] and used the *ad hoc* statistic ΔK developed by Evanno *et al.* (2005). STRUCTURE HARVESTER version 6.8 (Earl and von Holdt, 2011) was used to process STRUCTURE results and to select the most likely K using the methods mentioned above. We plotted the ranked partial membership of each individual to each cluster inferred (the membership coefficient - q value) in order to detect which samples were not clearly assigned (Beaumont *et al.*, 2001; Bergl and Vigilant, 2007). For each inferred cluster, we estimated the genetic diversity (H_o and H_e) and the pairwise F_{st} (Weir and Cockerham, 1984), using GENETIX version 4.05 (Belkhir *et al.*, 1996-2004).

We next used a non-spatial and spatial clustering of individuals in BAPS (Corander *et al.*, 2006). We used 5, 10 and 15 as priors for K repeated five times. We performed 10 independent runs to assess repeatability of results.

Finally, we used GENELAND v3.3.0 (Guillot *et al.*, 2005) for comparison of the results obtained by the BAPS spatial analysis. This analysis was run 10 independent times, assuming K to be between 1 and 10, assuming non-correlated and correlated allele frequencies and accounting for the presence and absence of null alleles. In the advanced options, the maximum number of nuclei was set to 447 (as recommended, three times the samples number) and the maximum rate of the Poisson process was fixed at 149 (corresponding to the samples number). Each run lasted for 100,000 iterations, with a thinning value of 1,000. We repeated the analysis for several degrees of GPS (Geographic Positioning System) uncertainty, varying between 0 and 4 Km (the maximum distance for which we could assure independence of social units) and did not find differences in the results for non-correlated alleles frequencies. Therefore, we only show the analysis using 4 Km as GPS uncertainty. After determining the most likely number of populations, we assigned each individual to each inferred cluster. We re-ran the analysis assuming non-

correlated allele frequencies and performed five independent runs using the parameters set established in the initial run.

4.3.3.2.2 - Mitochondrial DNA

Analysis of molecular variance (AMOVA, Excoffier *et al.*, 1992) was performed within social units, between social units within each sampling region and between sampling regions, using Arlequin version 3.11. The correlation between genetic differentiation and geographic distance was investigated using a Mantel test and by a spatial autocorrelation analysis, both performed using ALLELES IN SPACE (AIS) (Miller, 2005). Significance of correlations was tested by permutation. In this analysis, the average genetic distance between individuals (A_y) was estimated per distance class. A_y varies between zero (where all individuals are identical) and one (all individuals are different). The difference between the mean across all distances classes and the A_y for each distance class was tested by a randomisation test (Miller, 2005).

The Bayesian approach implemented in BAPS “clustering with linked loci” analysis (Corander *et al.*, 2008a; Corander and Tang, 2007) was used to infer homogenous groups of haplotypes. As priors for K, we used 5, 10 and 15, repeated 5 times. We performed 10 independent runs to assess repeatability of results.

4.3.3.3 - Migration and landscape factors influencing population structure

To identify migrants we used two different Bayesian approaches. STRUCTURE was run with the option USEPOPINFO to compute the probability that an individual belonged to a sampled genetic cluster. We divided samples into two groups Cantanhez + Cufada and Boé (see results) and ran the analysis three times, assuming migration rate (MIGPRIOR) to vary between 0.001 and 0.05 and GENSBACK to vary between 0 and 3. Burn-in and MCMC length were the same for the previous analysis. Additionally, we used GENECLASS version 2.0 to detect first generation migrants (Piry *et al.*, 2004). In this method, two likelihood-based tests statistics are estimated: L_h (more adequate for when the source population might not have been sampled) and L_h/L_{max} (better for when all source populations were presumably sampled) (Paetkau *et al.*, 2004). The Rannala and Mountain (1997) Bayesian method and the Paetkau (2004) resampling Monte Carlo algorithm (1,000 simulations and an alpha level of 0.01) were used to identify the critical values distinguishing between residents and migrants.

To infer geographic location of possible barriers to dispersal, we first used Monmonier's algorithm, implemented in AIS (Miller, 2005) for both genetic markers. Additionally, we used the interpolation function implemented in QGIS (inverse distance weighted, IDW) to detect genetic discontinuities and thus detect which features of the landscape might hinder dispersal (Manel *et al.*, 2003). We compared the information given by the two genetic markers. To perform the interpolation for microsatellite loci we used the individual q membership coefficient inferred by STRUCTURE. The interpolation using mitochondrial DNA data was performed using the proportion of individuals within each social unit assigned to the clusters inferred by BAPS (see above).

4.3.3.4 - Bushmeat samples assignment

To determine the most probable origin of the bushmeat samples, we used a two-fold approach. Firstly, we constructed a reference dataset dividing the individuals by sampling locations (Cantanhez, Cufada and Boé) and ran an assignment analysis using the “trained clustering of individuals” module in BAPS v5.0 (Corander *et al.*, 2006; Corander *et al.*, 2008a). The maximum number of K was set as 5, 10 and 15, repeated 5 times. Ten independent runs were performed to assess convergence of results. This analysis uses reference datasets defined by the user (in our case, the samples collected at each sampling location) and allocates samples of unknown origin to the most probable location, although as a result of this analysis, new clusters can be formed (Corander *et al.*, 2006; Corander *et al.*, 2008a). In the second approach we used the Rannala and Mountain (1997) Bayesian method, with MCMC re-sampling (Cornuet *et al.*, 1999), using 10,000 simulated individuals and 0.01 Type I error probability, as implemented in GENECLASS v2.0 (Piry *et al.*, 2004).

4.4 – Results

4.4.1 - DNA Extraction and Amplification

Out of the faecal samples extracted, 149 genotypes were included with a QI varying between 0.55 and 1 (averaging 0.83), along with the 14 tissue samples, successfully typed for all loci. Average amplification success across loci was of 80.8 %, ranging between 59 % (D1S533) and 94 % (D8S1106 and D6S501) (Table 1).

Of the samples genotyped we distinguished 175 different individuals. Repeated individuals (n = 45) were mainly found within social units with the exception of two

samples from the same individual collected in the Bubatchingue and Guebombol social units. The probability of identity (pID) using this set of loci was of $2,28 \times 10^{-9}$ for unrelated individuals and among sibs (pIDsib) was of 1.45×10^{-4} , which in principle allowed discrimination of individuals with only seven loci.

Micro-Checker analysis pointed to a heterozygote deficiency due to the presence of null alleles when all samples were pooled and when the samples were analysed according to their localities for D14S306 in Cantanhez samples and D2S1326 and D1S533 in Boé. No evidences of stuttering errors or large allelic dropout were found. Three loci were not in HW equilibrium when all samples were pooled together (D12S375, D2S1326, D1S533). We found evidences of linkage disequilibrium when samples were analysed by sampling regions (Cantanhez 9 pairs, Cufada 10 pairs and Boé 4 pairs). After Bonferroni adjustment for multiple comparisons, only one pair in Cantanhez and four pairs in Cufada remained in significant LD ($p < 0.0036$).

High quality mitochondrial (mtDNA) sequences were obtained for 165 of the 175 different individuals distinguished. We achieved 86 mtDNA sequences for the Cantanhez region, 56 sequences for Cufada and 23 sequences for Boé region. Additionally, we successfully amplified and sequenced for the aforementioned mtDNA fragment 4/14 tissue samples and 6 hair samples collected from captive individuals.

4.4.2 - Genetic Diversity

4.4.2.1 - Microsatellite loci

The fourteen loci typed had an average of 4.24 alleles per locus, UHe, averaged at 0.58, ranging between 0.55 (Cantanhez) and 0.61 (Boé) (Table 4.3). Mean number of alleles per locus did not vary between sampling regions nor did allelic richness. H_o was lower than H_e , predominantly in the Boé region, the sampling area that also shows significantly positive *Fis* values and two loci in HW disequilibrium ($p < 0.01$) (Table 4.3). Pairwise *Fst* values pointed to greater differentiation between Boé and the other sampling locations (Table 4.4).

4.4.2.2 - Mitochondrial DNA

The 393 bp fragment contained 55 polymorphic sites, with 56 substitutions and 7 sites with alignment gaps. Forty-one haplotypes were found (or 39 if gaps were not

considered). Mean haplotype ($Hd \pm SD$) and nucleotide ($\pi \pm SD$) were relatively high ($Hd = 0.81 \pm 0.024$; $\pi = 1.3 \% \pm 0.0011$). The Boé region showed the greatest haplotype diversity ($Hd_{Boé} = 0.91 \pm 0.04$, $n = 13$ haplotypes) and nucleotide diversity ($\pi_{Boé} = 2.1\% \pm 0.002$), followed by Cantanhez ($Hd_{Cantanhez} = 0.79 \pm 0.03$, $n = 21$ haplotypes; $\pi_{Cantanhez} = 1.04 \% \pm 0.002$) and Cufada ($Hd_{Cufada} = 0.68 \pm 0.061$, $n = 15$ haplotypes; $\pi_{Cufada} = 1.11 \% \pm 0.002$). Pairwise *Fst* based on haplotype frequencies was significant between all sampling regions (Table 4.4) and larger between Boé and Cantanhez and Cufada than between Cantanhez and Cufada. Nevertheless, when considering differentiation between sampling regions based on pairwise differences, Cantanhez and Cufada are not significantly different but Boé is significantly differentiated from Cantanhez and Cufada (Table 4.4).

Table 4.3: Genetic diversity statistics for Microsatellite loci and mtDNA. Expected Heterozygosity (He), Unbiased expected heterozygosity $\pm SD$ (UHe), observed heterozygosity $\pm SD$ (Ho), average number of alleles (Na), Allelic Richness (AR) based on 10 individuals, inbreeding coefficient (*Fis*) and number of loci in Hardy Weinberg (HW) disequilibrium, Pairwise *Fst* values based on number of different alleles (*denotes significant values). mtDNA: nH: number of haplotypes; Haplotype diversity (Hd) and Nucleotide diversity (π). Overall line indicate mean figures including tissue and hair samples. Average number of pairwise differences of haplotypes found within populations.

Sampling region	Microsatellite loci							mtDNA			
	Hexp	UHe	Hobs	Na	AR	Fis	HW	nH	Hd	π	Pairwise
Cantanhez	0.55 ± 0.13	0.56 ± 0.13	0.55 ± 0.13	4.21	3.43	0.017	0	21	0.797 ± 0.03	1.04% ± 0.002	4.32 $\pm 2,2$
Cufada	0.59 ± 0.11	0.59 ± 0.12	0.60 ± 0.12	4.23	3.65	-0.005	0	15	0.681 ± 0.004	1.11% ± 0.002	4.83 $\pm 2,4$
Boé	0.60 ± 0.09	0.61 ± 0.09	0.53 ± 0.17	4.29	3.88	0.138* ($p < 0.01$)	2	13	0.91 ± 0.04	2.1% ± 0.002	9.03 $\pm 4,3$
Overall	0.58	NA	0.56	4.24	3.65	NA	NA	41	0.81 ± 0.024	1.3% ± 0.001	NA

Table 4.4: Pairwise *Fst* between sampling regions using microsatellite loci and mtDNA. The number of different alleles was selected for microsatellite loci and haplotype frequencies (Haplotype column) and pairwise differences for mitochondrial DNA (Pairwise column). * Denotes significance ($p < 0.05$).

Pairwise Fst	Microsatellite loci		mtDNA			
	Cantanhez	Cufada	Cantanhez		Cufada	
			Haplotype	Pairwise	Haplotype	Pairwise
Cufada	0.017*		0.043*	0.018		
Boé	0.084*	0.038*	0.17*	0.257*	0.13*	0.356*

4.4.3 - Genetic Structure

4.4.3.1 - Microsatellite loci

AMOVA based on the number of different alleles revealed greater variation within social units (95.1 %, $p < 0.001$) than between social units within the same sampling region (1.6 %, $p < 0.001$). Variation between sampling regions was also small (3.34 %, $p < 0.001$).

STRUCTURE analysis, assuming either independent or correlated alleles frequencies, pointed to the presence of two genetic units when using the ΔK method (Evanno *et al.*, 2005) (Fig. 4.2a). While $K = 2$ was the highest modal value in the ΔK distribution, a second elevated modal value appeared when K was five (Fig. 4.2a) (more pronounced when considering uncorrelated allele frequencies, result not shown). The clustering solution with the largest Log(likelihood) and posterior probability across all runs was $K = 3$ (posterior probability_{K3} = 1) (either using non-correlated and correlated allele frequencies). When $K = 3$ visual inspection of the STRUCTURE plots showed that clusters 1 and 2 comprised the same individuals just belonging to cluster 1 when $K = 2$.

Individual q membership when $K = 2$ was similar between runs and so, for the following analysis, the mean of individual q values between runs was calculated. After plotting the ranked individual q values, following the approach of Beaumont *et al* (2001), we could not detect a clear pattern, and q values were nearly continuous (not shown). Therefore, we used an arbitrary threshold of $q > 0.80$ (Beaumont *et al.*, 2001; Vaha *et al.*, 2007) to assign the individuals to each cluster when and all other individuals (0.2-0.8) were treated as a product of admixture between clusters. Not including the tissue samples (see below), 60 individuals were assigned to cluster 1, 25 individuals were assigned to cluster 2 and 64 individuals were considered admixed. The Boé region predominately comprised by individuals with $q > 0.8$ in cluster 2 (68 % of individuals) whereas in Cantanhez, individuals assigned only to cluster 1 (61% of individuals) and admixed individuals (38 %), were present (Fig. 4.2c). Cufada, however, seems to represent an area of contact between clusters. Although the majority of individuals sampled in Cufada were admixed between clusters (53% of individuals), a relatively high proportion of individuals with a $q > 0.80$ to either of the clusters are also present (Fig. 4.2c). Almost twenty-eight percent of individuals in Cufada was assigned to cluster 1 and 19 % of individuals were assigned to cluster 2. Individuals with higher q membership to cluster 2 were present in all social units in Cufada

with the exception of Sr. Soares 2 (social unit labelled as 14 in Fig. 1), which is mainly constituted by individuals assigned to cluster 1 and admixed individuals (Fig 4.2c).

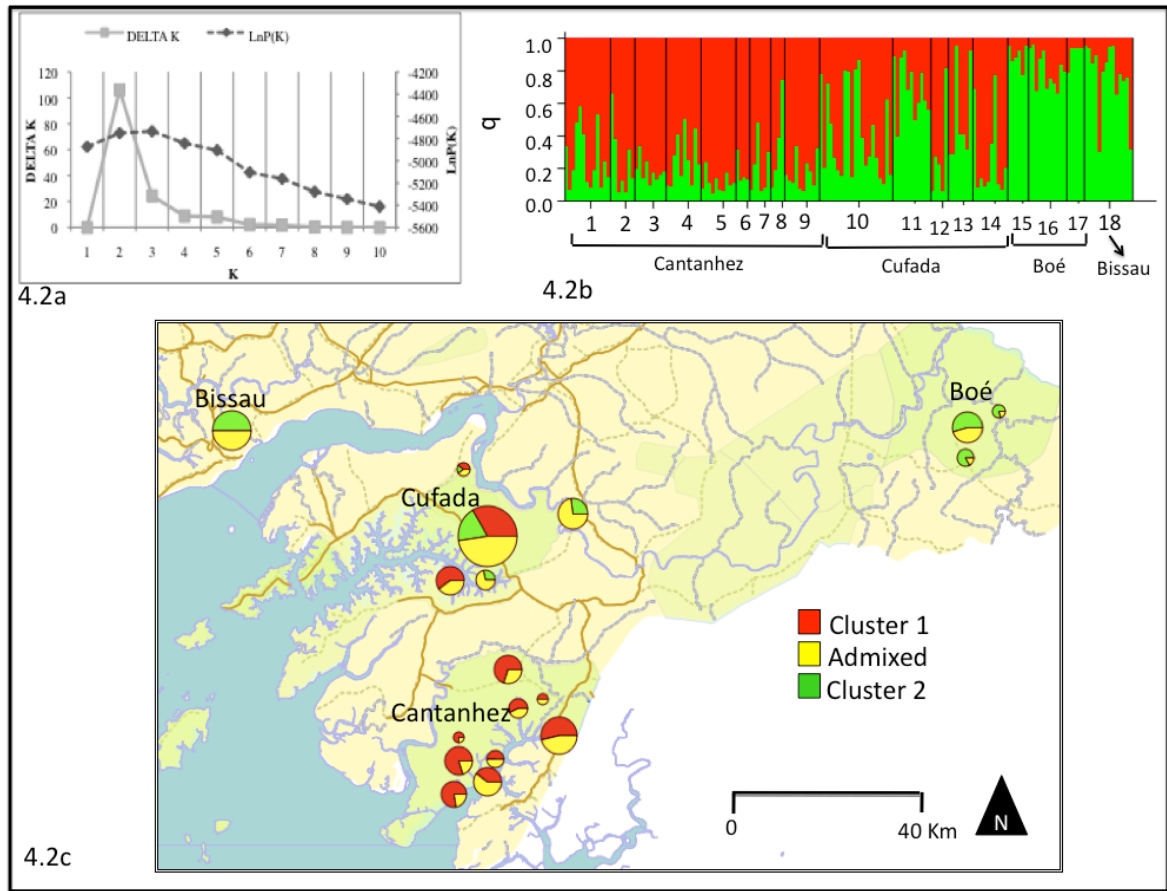


Figure 4.2: Population structure as inferred by STRUCTURE Fig. 4.2a shows DELTA K and Ln P(K) values as a function of number of putative populations (K); Fig. 4.2b indicates best partition when K = 2. Figure shows q membership of individuals in the two clusters. A single vertical bar represents each baboon and the code of social unit where the individual was sampled is indicated below. Cantanhez: 1 - Porto Gadamael; 2 - Amindara Catobo; 3 - Cabedu; 4 - Catomboi; 5 - Canamina; 6 - Caiquene; 7 - Cambeque; 8 - Quebo-Sutuba; 9 - Botchê Cule; Cufada: 10 - Bubatchingue; 11 - Bakar Contê; 12 - Guebombol; 13 - Sr.Soares1; 14 - Sr.Soares2; Boé: 15 - Boé Beli; 16 - Boé Aicum; 17 - Boé Montanha; 18 - bushmeat samples collected in Bissau. Fig. 4.2c show the proportion of each social unit assigned to each cluster ($q > 0.8$) and admixed between clusters (represented in yellow). Circles size is proportional to the sampling effort. Main road is indicated by a continuous brown line. Watercourses are indicated by blue lines (continuous line indicate main rivers and dashed lines represent smaller rivers).

The two genetic units uncovered by STRUCTURE were moderately differentiated (Pairwise $F_{st} = 0.15$, estimated using only the individuals with $q > 0.8$) and possessed different levels of genetic diversity. Individuals with a high ancestry to cluster 1 had lower genetic diversity than individuals assigned to cluster 2 ($He_{cluster1} = 0.51 \pm 0.14$ and $He_{cluster2} = 0.59 \pm 0.08$, respectively).

The non-spatial clustering of individuals in BAPS grouped individuals into a between five and eight clusters with posterior probabilities varying from 0.50 to 0.99 (not

shown). Spatial analysis in BAPS consistently pointed to the existence of two clusters (with a posterior probability varying between 0.88 and 1), a concordant result with the analysis performed in STRUCTURE (Fig. 4.3). Cluster 1, as inferred by BAPS spatial, was formed by all the individuals from Cantanhez and by 46 individuals from Cufada and Cluster 2 was formed by eight individuals from Cufada (distributed across all social units with the exception of Sr. Soares 2) and by all individuals from Boé. All individuals from Cufada assigned to cluster 2 by the STRUCTURE analysis ($q > 0.80$) except one were also assigned to cluster 2 by BAPS spatial.

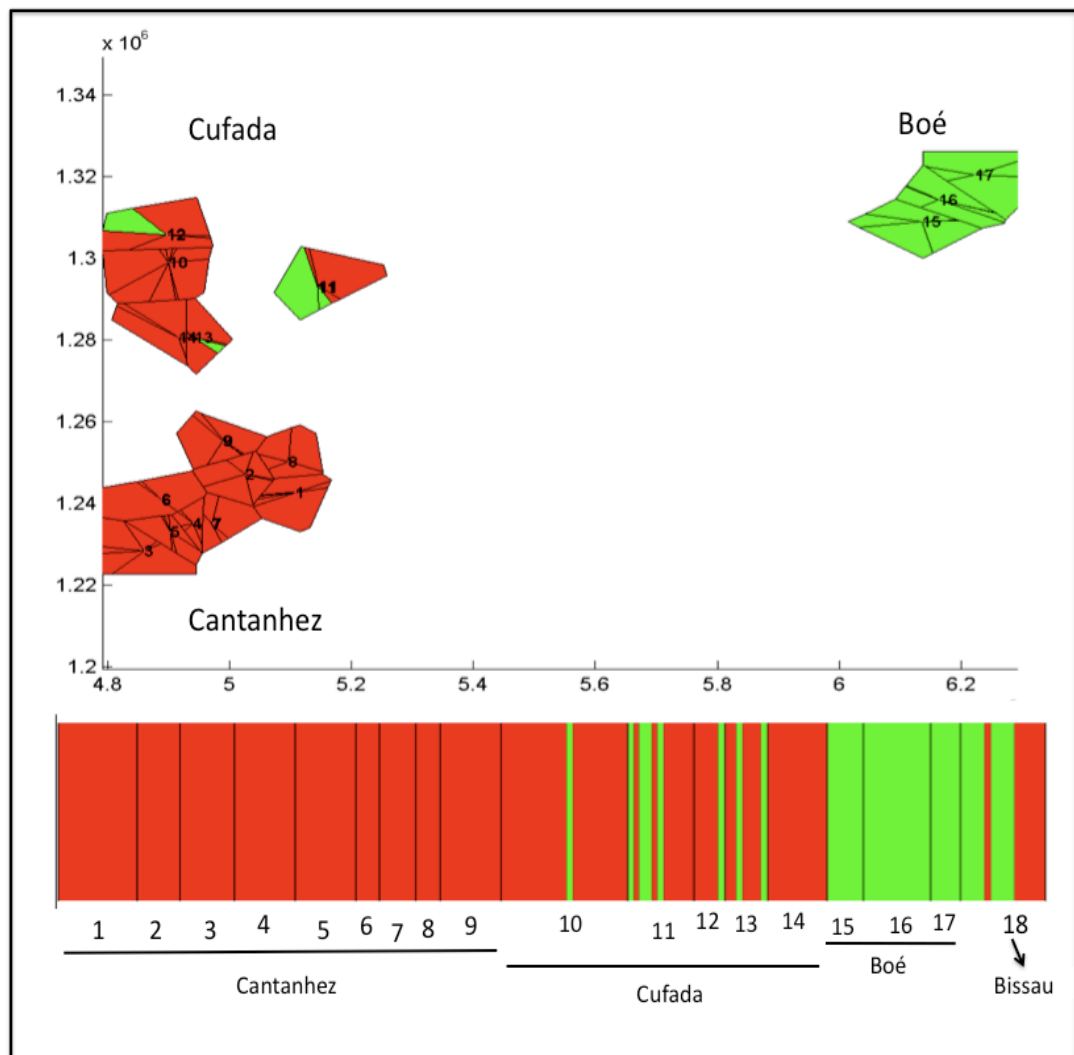


Figure 4.3: Spatial structure as inferred by BAPS. The spatial location of each sample is showed above. The different colours represent the clusters detected. Below, a single vertical bar represents each baboon and the code of social unit where the individual was sampled is indicated. Cantanhez: 1 - Porto Gadamael; 2 - Amindara Catobo; 3 - Cabedu; 4 - Catomboi; 5 - Canamina; 6 - Caiquene; 7 - Cambeque; 8 - Quebo-Sutuba; 9 - Botchê Cule; Cufada: 10 - Bubatchingue; 11 - Bakar Contê; 12 - Guebombol; 13 - Sr.Soaresh; 14 - Sr.Soaresh2; Boé: 15 - Boé Beli; 16 - Boé Aicum; 17 - Boé Montanha; 18 - bushmeat samples collected in Bissau.

The spatial analysis performed in GENELAND across all runs using non-correlated allele frequencies indicated the presence of two genetic clusters in all ten independent runs (Log posterior probability of -7558.5) (Fig. 4.4a and 4.4b). When the model was run to assign each individual to the two clusters, all individuals from Cantanhez along with 36 individuals from Cufada were assigned to cluster 1 and all individuals from Boé were assigned to cluster 2 (with a posterior probability of 1). Twenty individuals from Cufada presented higher posterior probability of assignment to cluster 1 but with a non-negligible posterior probability of assignment to cluster 2 (between 0.58 and 0.001, average 0.4). This is represented in GENELAND graphs with areas of steep turnover in posterior probabilities crossing the geographical locations of the Bakar Contê and Sr. Soares 1 social units (Fig. 4.4c). Five of the twenty individuals sampled in Cufada were also assigned to cluster 2 by STRUCTURE and six by BAPS spatial analysis.

When GENELAND was run considering correlated allele frequencies, not accounting for the presence of null alleles, the maximum number of populations increased to five (consistent across all runs, Log posterior Probability of -6867.2). Cluster 1 was formed by all individuals from Boé and a few individuals from Sr. Soares 1 and Bakar Contê social units (Cufada); cluster 2 was formed by all individuals from the Bubatchingue social unit; cluster 3 was formed by individuals from the Sr. Soares 1 and Bakar Contê social units (Cufada); cluster 4 was formed by all individuals from Cantanhez and two social units from Cufada (Sr. Soares 1 and Guebombol); and cluster 5 was formed by a few individuals from Porto Gandamael (Cantanhez). When considering correlated alleles frequencies and presence of null alleles in the dataset, the maximum number of populations varied between three (Log posterior Probability of -9751,2) and four (Log posterior Probability of -9991.0) (data not shown).

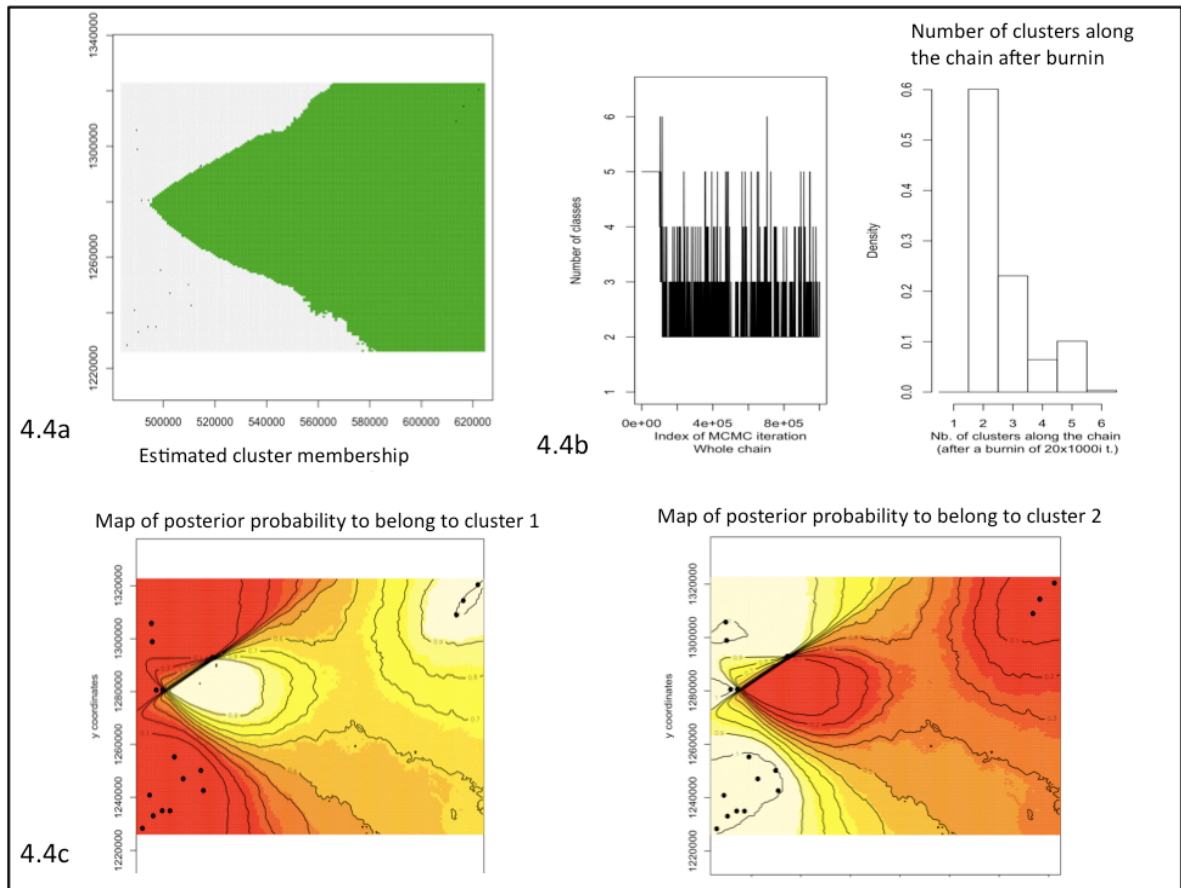


Figure 4.4: Spatial structure as inferred by GENELAND. Fig. 4.4a: The map of estimated population membership; 4.4b: the number of population simulated from the posterior distribution (most probable is $K=2$); 4.4c: the probability to belong to cluster 1 and cluster 2 (black dots point to the geographic location of each social unit and dark areas represent high values of probability)

Mantel tests pointed to a pattern of isolation by distance when all samples are combined ($R_{xy} = 0.374$, $p < 0.001$), although this significant linear correlation is rather weak ($R^2 = 0.14$). The spatial autocorrelation analysis illustrated the pattern uncovered by BAPS and GENELAND when all samples were pooled. Significant genetic similarity ($p < 0.05$) was found between 16.5 Km to 33 Km (broadly corresponding to the distances classes within sampling locations) and at 115.5 Km (corresponding roughly to the distance between the Boé social units and Bakar Contê social unit, approximately 110 Km) (Fig. 4.5). Pairwise genetic dissimilarity increased significantly ($p < 0.05$) at the distance class of 66 Km and at higher distances classes (132 – 165 Km) (corresponding to the distance between the Boé social units and the Cantanhez sampling locations) (Fig. 4.5).

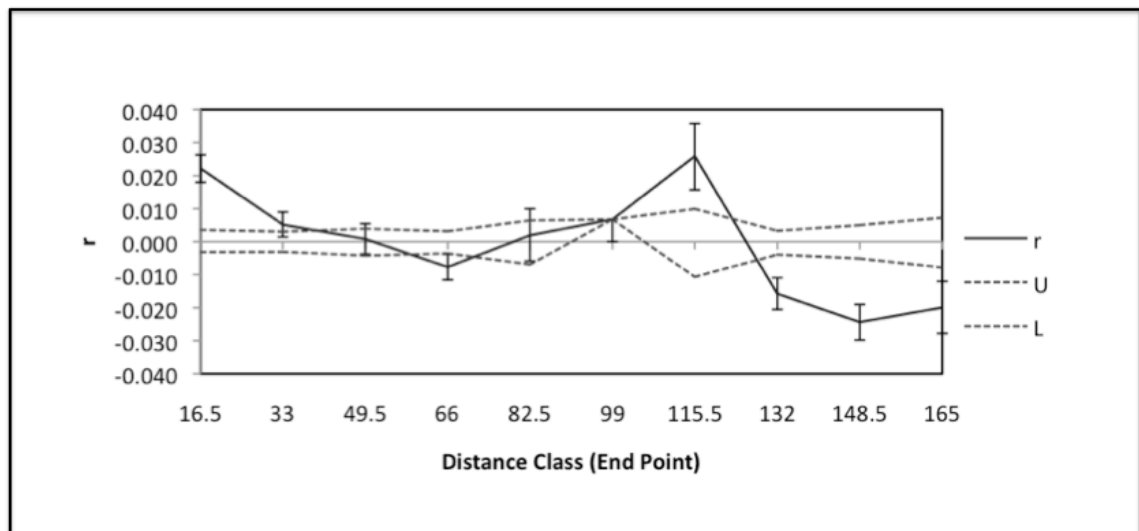


Figure 4.5: Autocorrelation analysis. Correlogram of the correlation coefficient (r) between genetic and geographic distance at ten distance classes (end point). U and L are upper and lower limits for the 95% confidence band under the null hypothesis of random distribution of genotypes across the landscape. Error bars represent 95% confidence intervals around each mean correlation coefficient. Distances classes (Km) with significant pairwise genetic distances are the ones standing outside the dashed lines.

4.4.3.2 - *mtDNA*

The network pointed to a lack of spatial structure for the distribution of haplotypes (Fig. 4.6). Four of the most frequent haplotypes H2, H1, H13 and H15, harboured by 60 % of individuals (36 %, 15 %, 4.8 % and 4.2 %, respectively), are shared between sampling locations. Each of these four haplotypes represents star-like phylogenies, where a more frequent haplotype is connected in the network by one mutational step to less frequent haplotypes (represented by one or two individuals) (Slatkin and Hudson, 1991). Moreover, just three haplotypes are shared between Cantanhez and Cufada (H4, H9 and H16), just one haplotype is shared between Cantanhez and Boé (H3) and just one haplotype is shared between Cufada and Boé (H28). In contrast, region-specific haplotypes for each of the sampling regions were also found (14 haplotypes in Cantanhez, nine haplotypes in Cufada and seven haplotypes in Boé), mostly represented by a single individual.

AMOVA based on haplotype frequencies revealed that most of the variation was present within social units (88.1 %, $p < 0.001$) compared to social groups within sampling regions (3.64 %, $p = 0.03$) and only 8.7 % ($p < 0.001$) of the total variation was explained by the sampling regions. However, when the AMOVA analysis was conducted based on the pairwise haplotypes differences, the percentage of variation explained by sampling regions increased to 17.24 %, due to the presence of very divergent haplotypes in the Boé region.

The percentage of variation within social units (79.97 %, $p < 0.001$) and between social units within sampling regions (2.78 %, $p = 0.1$) decreased slightly.

Mantel test and spatial autocorrelation analysis failed to determine any significant linear correlation between genetic distance and geographic distance (Mantel test: 1,000 permutations, $r = 0.051$, $p = 0.057$; Autocorrelation analysis: 10,000 permutations; average $A_y = 0.014$, $V = 0.0012$, probability of observing a value greater or equal to V by chance of 0.1210) when all samples were pooled. On the other hand, in the spatial autocorrelation analysis, the pairwise genetic distances were significantly different from the mean at two distances classes (Fig. 4.7): at 16.5 Km ($A_y = 0.013$, $p = 0.01$), where individuals were significantly dissimilar and at 148.9 Km ($A_y = 0.0164$, $p = 0.001$), where individuals were significantly similar, the later corresponding to geographical distance between Boé and Cantanhez (Fig. 4.7). We did not find a significant correlation between genetic and geographic distance when only the Cufada and Cantanhez samples were analysed ($r = 0.043$, $p = 0.12$) or when only Cantanhez and Boé samples were considered ($r = 0.051$, $p = 0.087$). For samples from Cufada and Boé we found a nearly significant correlation ($r = 0.084$, $p = 0.058$).

Bayesian analysis performed in BAPS divided haplotypes into six clusters (with a posterior probability of 1, consistent across runs) (Fig. 4.8): Cluster 1 was represented by four individuals; Cluster 2 was represented by 47 individuals; Cluster 3 was formed by 12 individuals; Cluster 4 was formed by three individuals; Cluster 5 was formed by 98 individuals and Cluster 6 was formed by 11 individuals (Fig. 4.8).

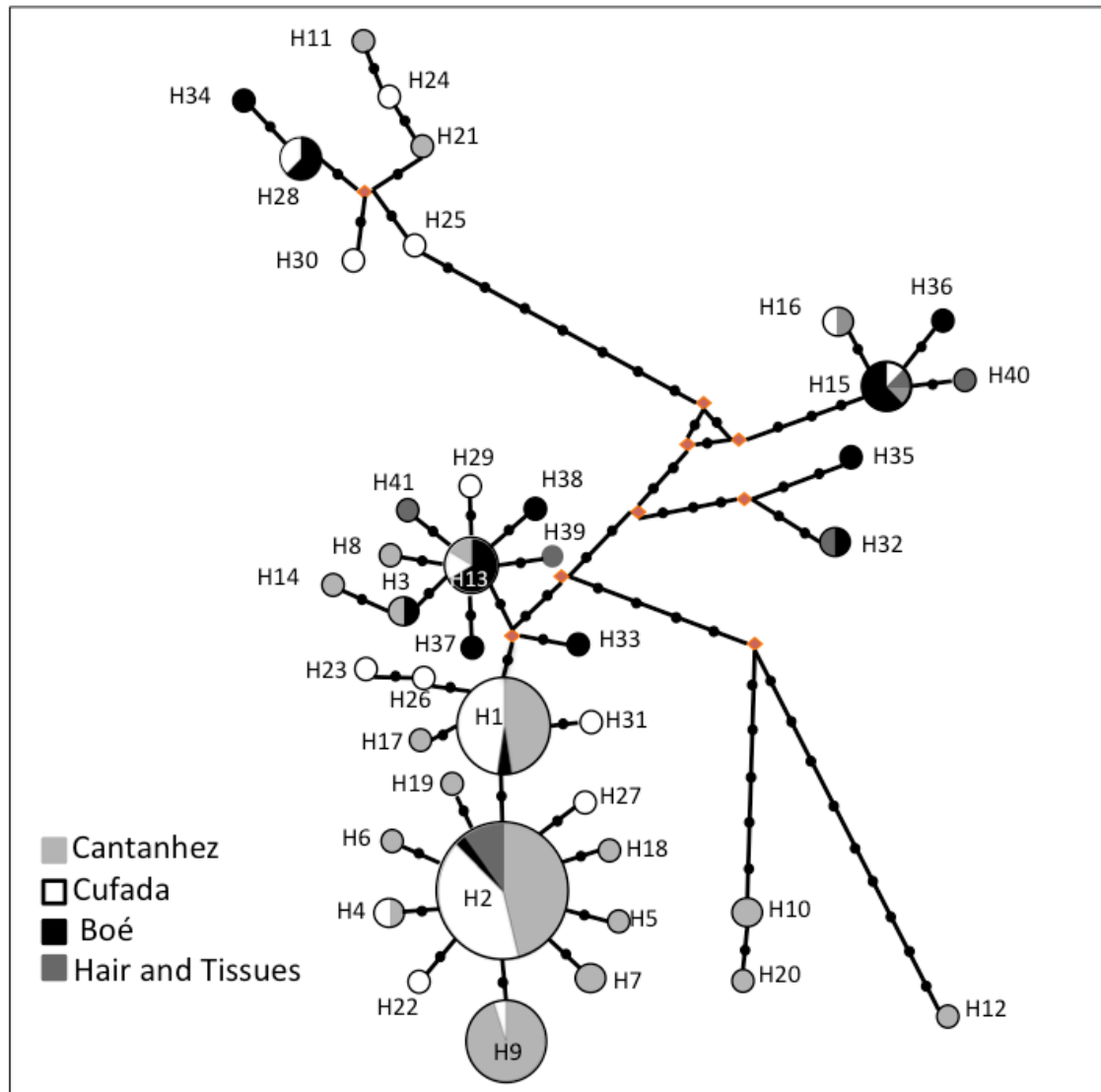


Figure 4.6: Median joining network showing the relationships among haplotypes based on a 393bp fragment of the mtDNA control region. The circles sizes are proportional to number of individuals harbouring the haplotype and branches lengths are scaled according with number of mutations that separate the haplotypes. Colours indicate sampling locations (Cantanhez-light grey, Cufada-white and Boé-black, tissues and hair samples-dark grey). Small squares (median vectors) represent not sampled haplotypes and small black circles indicate the characters where a mutation is present.

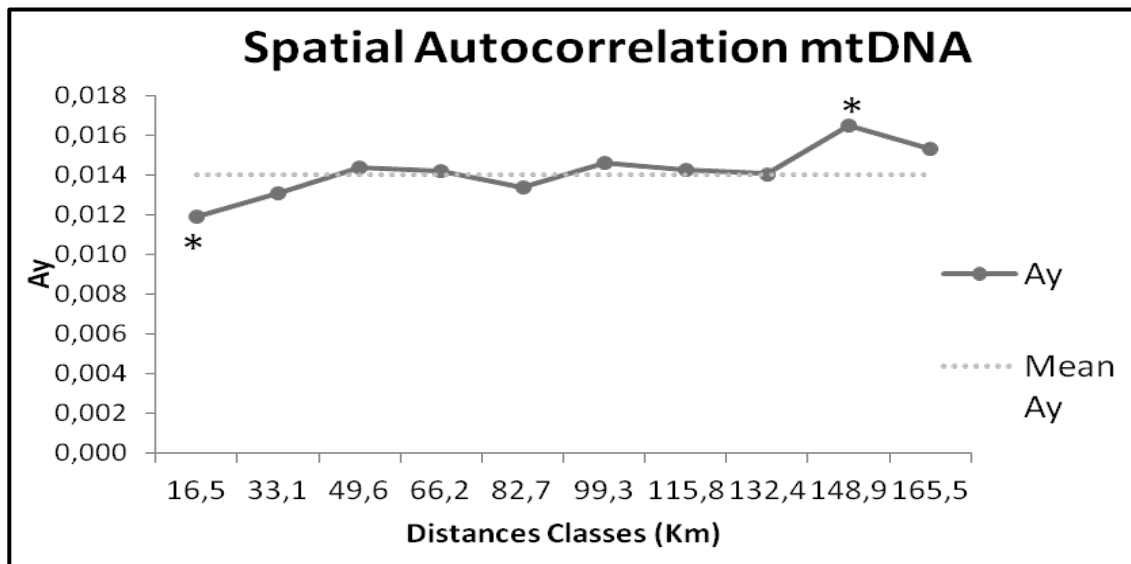


Figure 4.7: MtDNA spatial autocorrelation analysis. The average genetic distance between individuals in distance class (Ay) is indicated by the dashed line on the graph. Ay values significantly different from the mean ($p < 0.05$) are indicated by *.

Sampling regions differ in the proportion of individuals assigned to each cluster (Fig. 4.9). The Boé region ($n = 23$) was mostly represented by cluster 2 (43.5 %), then cluster 3 (26.1 %) and finally cluster 6 (17.4 %). Cluster 5 is the least represented (4.34 %), contrasting with the number of individuals assigned to this cluster in Cantanhez and Cufada. Cluster 4 was only represented in Boé region (by 2 individuals) and by one individual living as a pet in Bissau (Fig. 4.9a). In Cantanhez ($n = 86$), individuals were mainly assigned to cluster 5 (69.8 %) and cluster 2 (20.9 %). Cluster 3 and 6 and were the least represented (2.33 %): cluster 3 by two individuals from Quebo-Sutuba social unit and cluster 6 by one individual from Catomboi and one individual from Cabedu. Haplotypes from cluster 1 were only found in Cantanhez (4 individuals) (Fig. 4.9a). The Cufada region ($n = 56$) showed a similar pattern, with most of the individuals assigned to cluster 5 (58.9 %) and cluster 2 (28.6 %) and only 3.57 % of the individuals were assigned to cluster 3 (one individual from the Sr. Soares 2 and one individual from Bakar Contê). Cluster 6 had a higher representation in Cufada than in Cantanhez (nearly 9% of individuals in Cufada, three individuals from Sr. Soares 1 and two individuals from Bakar Contê) (Fig. 4.9b).

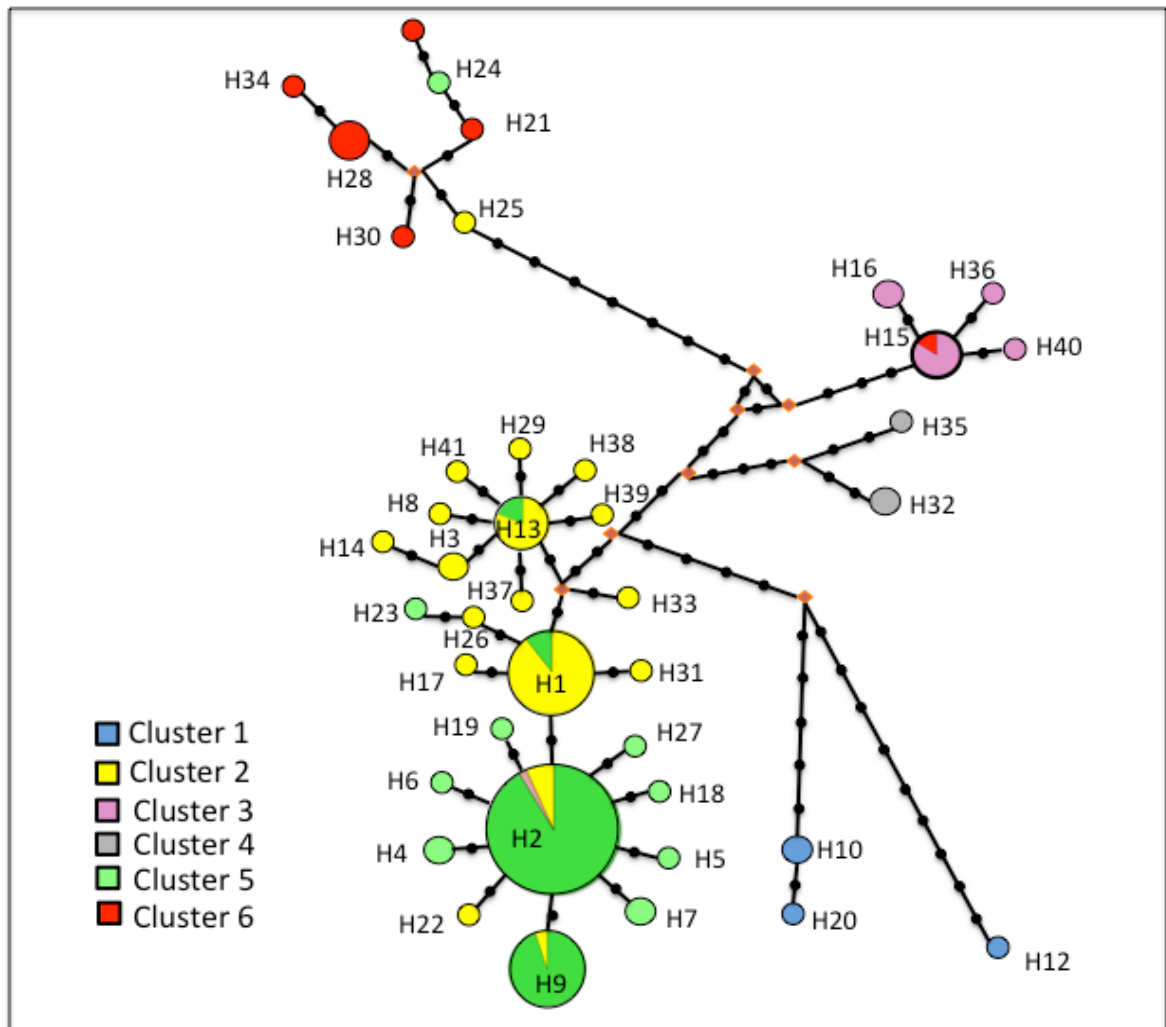


Figure 4.8: The six clusters of haplotypes assessed by BAPS clustering with linked loci indicated in the median joining network. Haplotypes Cluster 1 in blue, haplotypes Cluster 2 in yellow, haplotypes Cluster 3 in pink, haplotypes Cluster 4 in grey, haplotypes Cluster 5 in green and finally, haplotypes Cluster 6 in red)

4.4.4 - Migration between regions and possible barriers to dispersal

4.4.4.1 - Migration between regions

Previous analyses allow us to infer that Cufada is a contact area between Cluster 1 and Cluster 2. We were therefore interested to understand if the individuals sampled in Cufada assigned to Cluster 2 could be considered migrants from the Boé region. We used GENECLASS to detect migrants and STRUCTURE using the USEPOPINFO option.

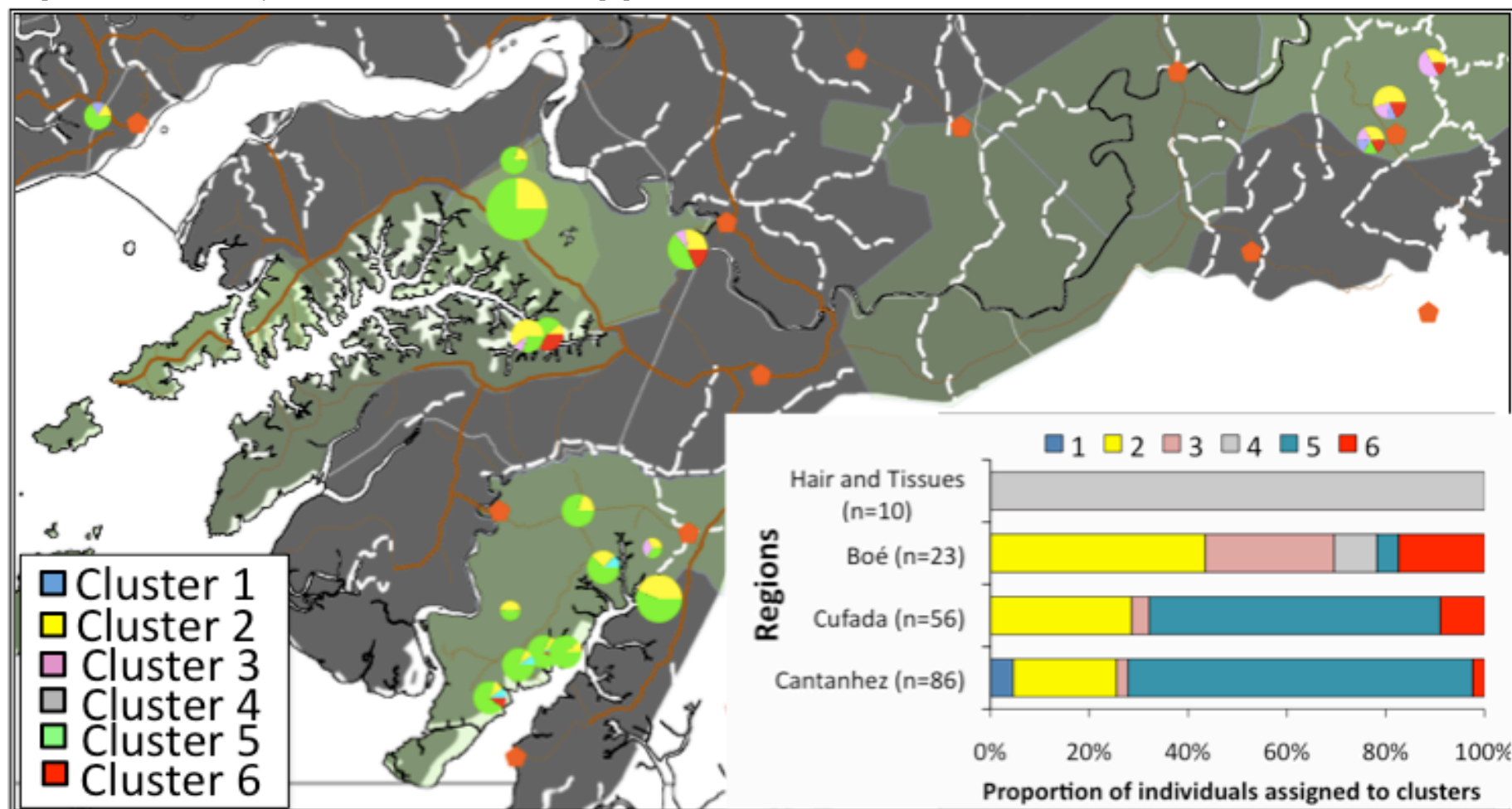


Figure 4.9: Map with the proportion of social groups harbouring haplotypes from each cluster. Main anthropogenic features are represented: main villages and road are indicated by orange pentagons and by a continuous brown line, respectively and small watercourses are represented by white dashed lines. Below a graph showing the proportion of individuals assigned to each cluster by regions Cantanhez in blue, Cufada in red, Boé in green and hair and tissue samples in purple.

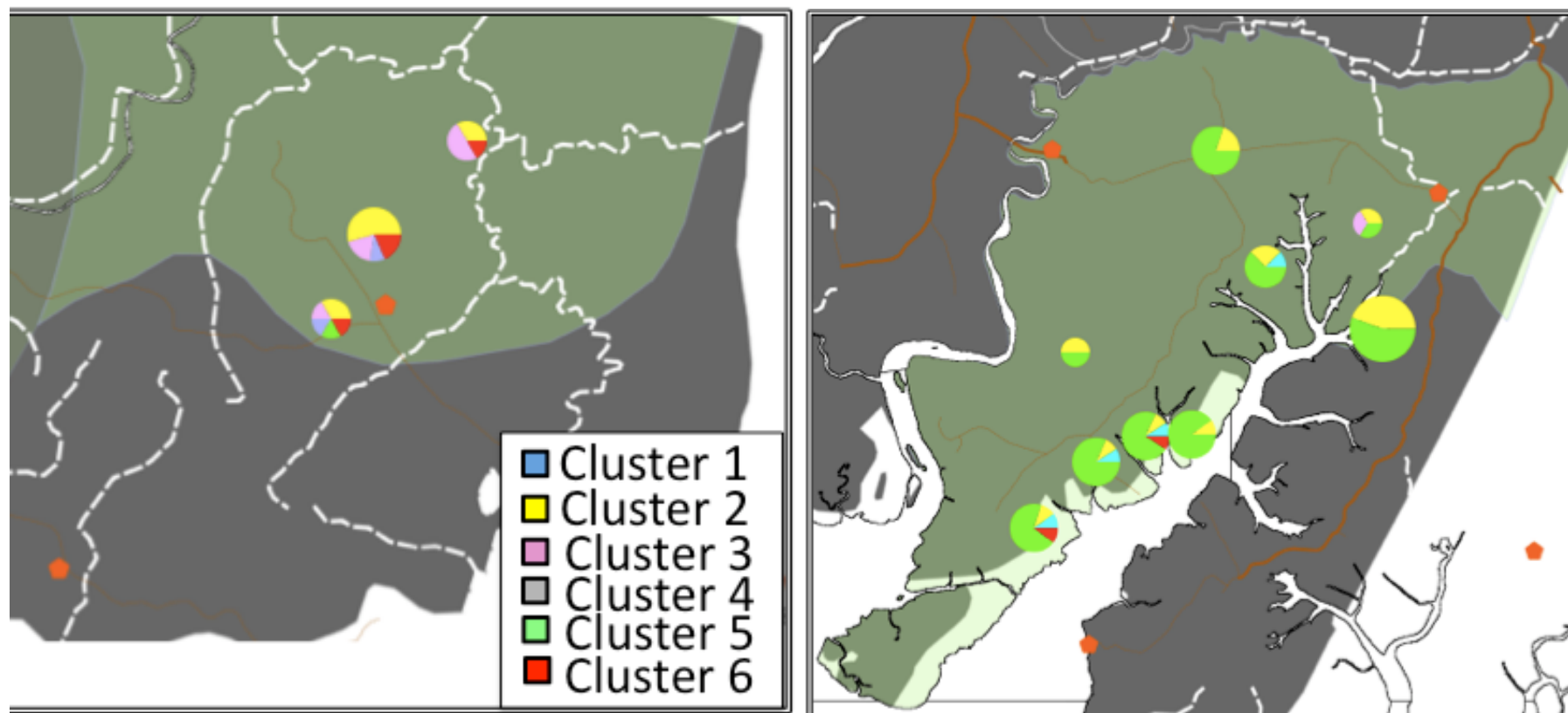


Figure 4.9a: Detail of Fig. 4.9 showing map with the proportion of social groups harbouring haplotypes from each cluster in Boé (left) and Cantanhez (right). Main anthropogenic features are represented: main villages and road are indicated by orange pentagons and by a continuous brown line, respectively.

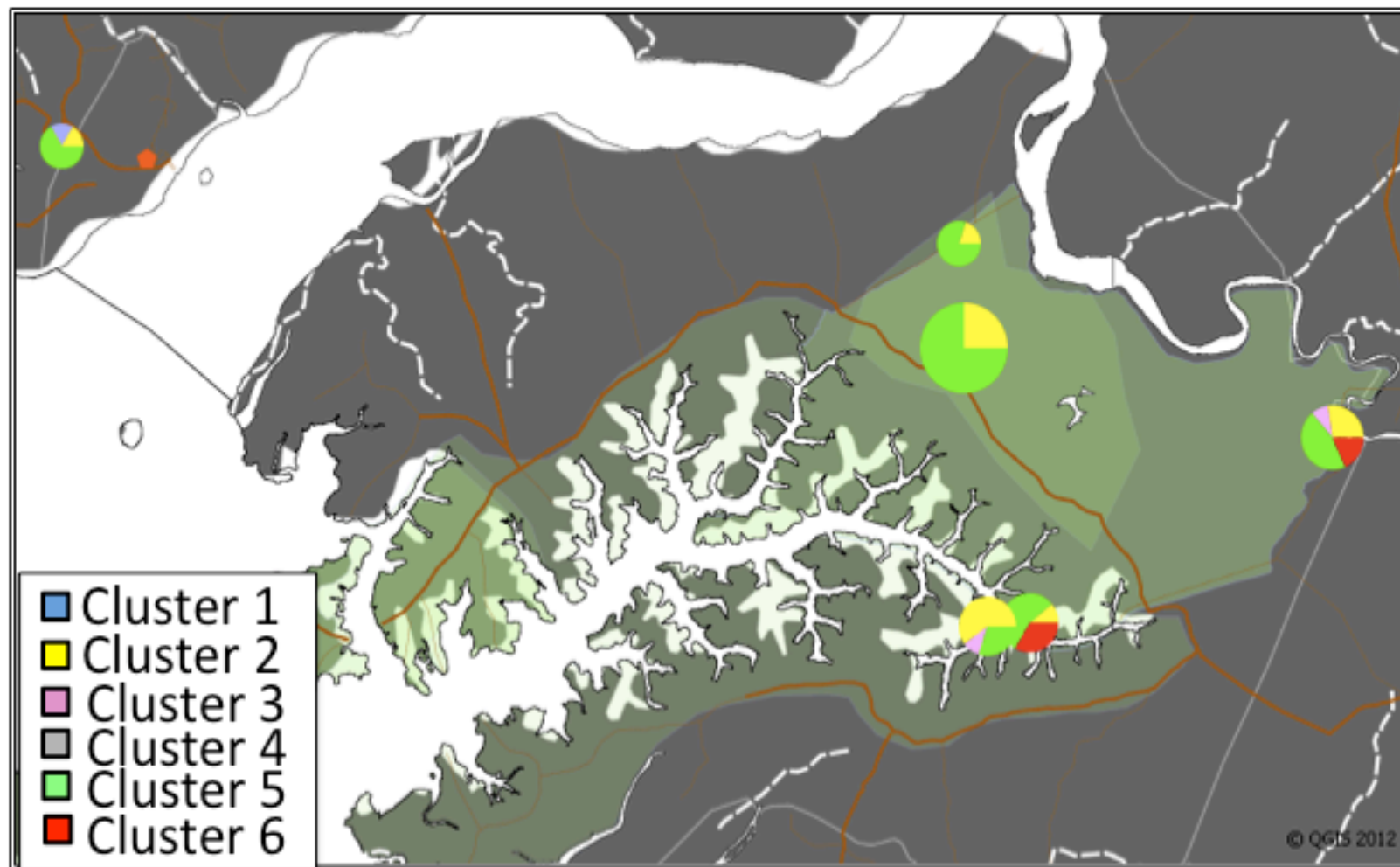


Figure 4.9b: Detail of Fig. 4.9 showing map with the proportion of social groups harbouring haplotypes from each cluster in Cufada and Bissau. Main anthropogenic features are represented: main villages and road are indicated by orange pentagons and by a continuous brown line, respectively and small watercourses are represented by white dashed lines.

The analyses run in GENECLASS identified six individuals as migrants using the L_h/L_{max} statistic: five sampled from Cufada with an origin in Boé (CUBAK1234, CUBAK1236, CUBAK1244, CU1SOA1307, CU1SOA1324) and one sampled from Boé with an origin in Cufada (BOAIC27) (Table 4.5). CU1SOA1307 was the only individual identified as a migrant using L_h method. The putative migrants sampled from Cufada have been assigned to Cluster 2 by STRUCTURE without prior information on their sampling location ($q > 0.8$) and by BAPS spatial analysis (CUBAK1234, CUBAK1236, CUBAK1244, CU1SOA1307 and CU1SOA1324).

The analysis performed using STRUCTURE was concordant with GENECLASS for most of samples (with the exception of CUBUB1220, not identified by GENECLASS and BOAIC27 identified solely by GENECLASS) (Table 4.5). Using MIGPRIOR of 0.05, four individuals were identified as migrants (CUBAK1234, CUBAK1244, CU1SOA1307 and CU1SOA1324) and four were identified as having migrant ancestry (CUBUB1220, CUBAK1236, CUBAK1252 and CUGUE1292) (see Table 4.5 for details). When the analysis was repeated with a close-to-zero restrictive migration rate (MIGPRIOR=0.01), only one individual (CU1SOA1324) was identified as a migrant. The individual CU1SOA1324 harbours an mtDNA haplotype (H28) that is grouped in a cluster with a high representation in Boé region (Cluster 6) (see interpolation analysis section).

4.4.4.2 - Possible barriers to dispersal

Monmonier's algorithm using microsatellite loci data identified two barriers, separating the dataset in three groups. The first barrier was placed between two social groups in Boé (Boé Beli and other Boé social units, not shown). The second barrier divided samples in three groups: i) Boé samples + Bakar Contê and Sr. Soares 1, ii) Cantanhez and all other social units of Cufada area, including Sr. Soares 2; iii) Quebo-Sutuba social unit (Fig. 4.10a).

Table 4.5: Migrant detection analyses. Individuals sampled in Cufada and assigned to cluster 2, by either STRUCTURE (STRUC) or BAPS SPATIAL (BASP), are indicated. SIG (in bold) represents individuals with a significant probability ($p < 0.01$) of being migrants and NS represents individuals with a non-significant probability. STRUCTURE analysis is shown with two different migration rates (0.05 and 0.01). The probability of residency (%) is indicated in column 1, the probability of being a first generation migrant (%) (Probability of assignment to Cluster 2) is indicated in column 2, the probability of having a migrant ancestry is indicated in column 1st (one generation back) and 2nd (two generations back). The final classification (CLASS) Migrant (M) and Admixed (A) take into account the concordance of results between GENECLASS and STRUCTURE MIGPRIOR = 0.05.

STRUC	BASP	GENECLASS		STRUCTURE MIGPRIOR = 0.05				STRUCTURE MIGPRIOR = 0.01				CLASS
		Lh/Lmax	Lh	1	2	1 st	2 nd	1	2	1 st	2 nd	
1214		NS	NS	>90				>90				A
1215		NS	NS	>90				>90				A
1219		NS	NS	>90				>90				A
1220		NS	NS	50	18	17	13	>90				A
1234		SIG	NS	12	60	22	6	43	39	13	4	M
1236		SIG	NS	51	17	20	12	88	4	4	3	A
1244		SIG	NS	6	67	20	6	39	43	13	5	M
	1252	NS	NS	31	7	38	26	85	1	7	6	A
1292		NS	NS	46	7	31	16	88	2	6	4	A
1307		SIG	SIG	0	93	7	0	1	91	7	0	M
1324		SIG	NS	11	71	13	5	65	25	7	3	M

The shape of the second main barrier was recapitulated by the interpolation analysis, based on q value computed by STRUCTURE (Fig. 4.10b). This analysis pointed to four genetic discontinuities: i) between Bakar Contê + Sr. Soares 1 and all other social units from Cufada; ii) between the Cantanhez social units + Sr. Soares 2 and all other social units; iii) between the Boé social units and all other areas; iv) between the Quebo-Sutuba social unit and all other social units. These barriers were already pointed out by the GENELAND analysis, although with lower resolution.

Monmonier's algorithm using the mtDNA data distinguished one main barrier, isolating the Cabedu social unit from all other social units (Fig. 4.10c). When the interpolation analysis was repeated using the mtDNA data, a spatial genetic pattern consistent with the microsatellite dataset was revealed. The interpolation analysis of cluster 6 and cluster 3 (mainly represented in Boé region) showed two main genetic discontinuities (Fig. 4.10d). Using cluster 6, two genetic discontinuities were found: between i) Bakar Contê + Sr. Soares 1 + Boé samples and all other social units and between ii) Cabedu and

Canamina social units and all other social units. Using cluster 3, Quebo-Sutuba and Boé samples became isolated from all other social units. The interpolation analysis using proportion of social units to cluster 5 and cluster 2 revealed an inversed pattern, although the Sr. Soares 1 social unit also presented a high proportion of individuals assigned to cluster 5 (Fig. 4.10d).

This mtDNA spatial analysis highlighted the high proportion of very divergent haplotypes present in the Sr. Soares 1 (H28 and H30), Bakar Contê (H15), Quebo-Sutuba (H15 and H16), Cabedu (H11) and Canamina (H21) social units when compared with the surrounding groups. These results are in agreement with the spatial autocorrelation analysis, which showed significant pairwise dissimilarity at smaller and at higher distances.

4.4.5 - Bushmeat tissue samples geographic assignment

For ten bushmeat samples, we obtained concordance between methods of assignment to a specific sampling region. Five samples were assigned to the Boé region and five samples were assigned to the Cufada region (Table 4.6). Nevertheless, the probabilities of assignment to each sampling location were not high (never exceeding 61 %), reflecting that the source population might not have been sampled or that sampling regions are not sufficiently differentiated to allow confidence in the assignment (Table 4.6). For example, for four samples (t25, t26, t14, t12) the results were not concordant. Sample t25 and t26 were not assigned to any sampling region by the GENECLASS analysis, although the BAPS analysis pointed to Boé and Cufada, respectively and samples t14 and t12 were assigned to different locations by the two different methods (Cantanhez and Cufada for t14 and Boé and Cufada for t12). Taking into consideration the high proportion of admixed individuals in both Cantanhez and Cufada sampling regions, probably samples t14 and t26 were not assigned due to their degree of admixture between Cluster 1 and 2. In the same way, samples t12 and t25 show greater probability of membership to Cluster 2 and the analysis could not distinguish between Cufada and Boé as most probable source (see Table 4.6).

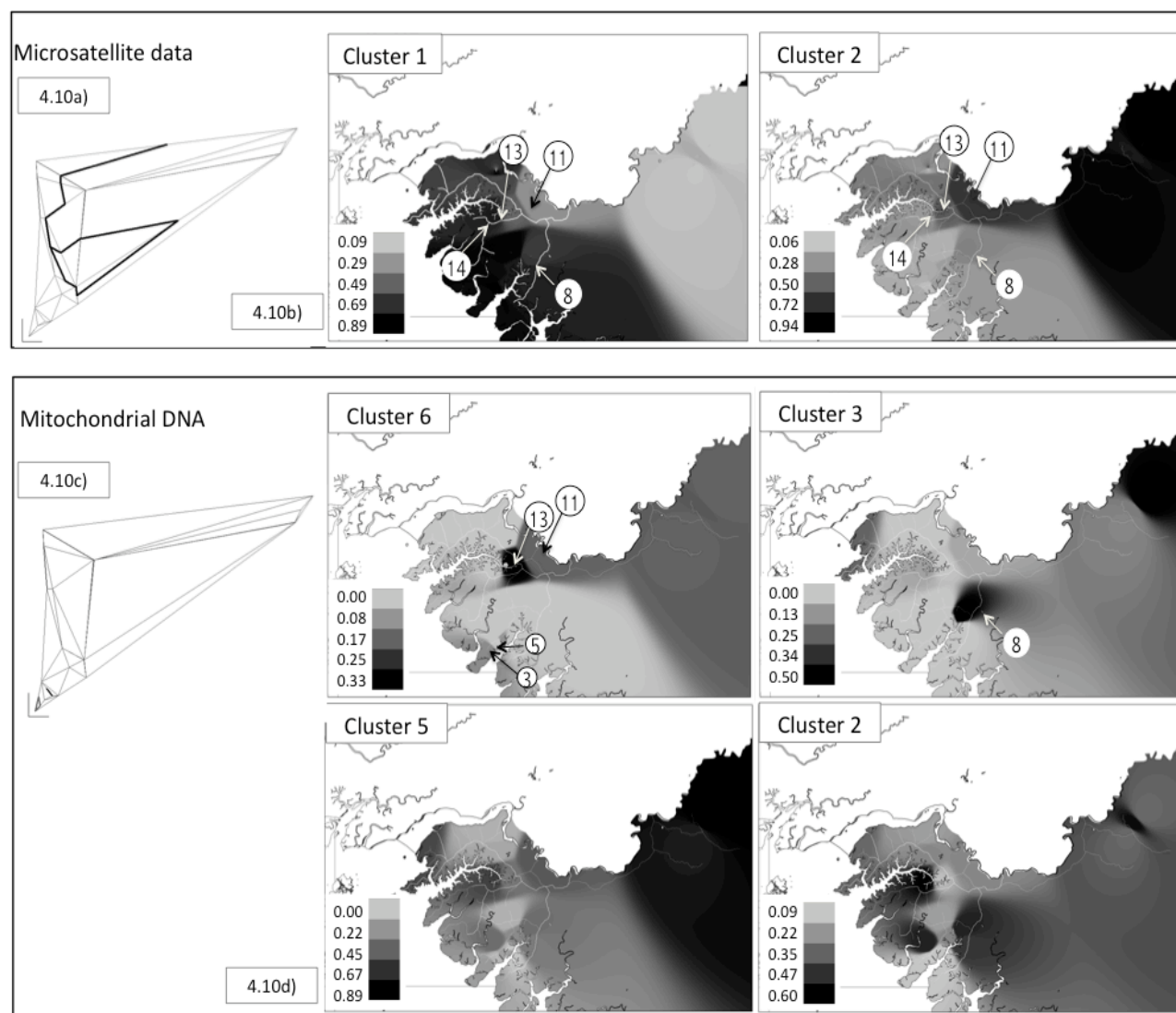


Figure 4.10: Genetic discontinuities. Fig. 4.11a: Second putative barrier as generated by Monmonier's algorithm using microsatellite loci data (corresponding to the second largest difference in genetic distances between individuals); 4.11b: Interpolation analyses for Cluster 1 and Cluster 2, using individual q values estimated by STRUCTURE analysis. q values were divided into five classes and legend shows mid-class point. Numbers indicate location of social units (8 – Quebo-Sutuba, 11 – Bakar Contê, 13 – Sr. Soares 1 and 14 – Sr. Soares 2). Fig. 4.11c: First putative barrier as identified by Monmonier's algorithm using mtDNA data (correspond to the first largest difference in genetic distance between individuals); Fig. 4.11d: Proportion of each social unit assigned to each of the clusters identified by BAPS using mtDNA data. Numbers indicate location of social units (3 – Cabedu, 5 – Canamina, 8 – Quebo-Sutuba). Note that Cluster 6 and 3 are occurring more frequently in the Boé region while Cluster 5 and 2 are more represented in the Cufada and Cantanhez sampling regions. Cluster 1 and 4 were not used as they are not shared between sampling locations, see results section. Genetic discontinuities were not concordant with the geographic location of human infrastructures (e.g. main roads are represented in the maps by a white continuous line).

The putative origin of bushmeat samples given by sellers at the bushmeat markets was initially thought to represent the origin of transportation and not the localities where individuals were hunted. Nevertheless, there is some concordance between the most probable origin identified by the genetic assignment test and the putative geographic location referred by bushmeat sellers. For example, samples whose origin would be closer to Cossé (t10 and t11) had a higher q membership to Cluster 2 (genetically closer to the Boé samples). In the same way, samples from Cacine (t41, t43, t54) were genetically closer to the ones from the Cufada and Cantanhez areas (either with higher q membership to Cluster 1 or admixture between clusters) (see Table 4.6).

Table 4.6: Results of the assignment tests to determine origin of bushmeat samples. The origin of samples referred by sellers is indicated as the putative origin. The q membership to each cluster, as computed by STRUCTURE, is indicated for comparison with the results obtained from trained clustering analysis performed using BAPS and the probability of assignment computed by GENECLASS. The concordant results between analyses are indicated in bold and non-concordant results are indicated in italic.

Bushmeat samples	Probability of assignment (%)			Trained Clustering	Putative origin	q membership	
	Cantanhez	Cufada	Boé			Cluster 1	Cluster 2
t10	0.0	6	9.6	Boé	Cossé	0.05	0.95
t17	0.0	1.2	6.9	Boé	unknown	0.06	0.94
t11	0.0	18.7	61.4	Boé	Cossé	0.07	0.93
t12	<i>1</i>	<i>3.1</i>	<i>2.5</i>	<i>Boé</i>	<i>Cossé</i>	<i>0.15</i>	<i>0.85</i>
t13	0.0	5.3	40.1	Boé	Xitole	0.10	0.90
t14	<i>14.1</i>	25	<i>0.0</i>	<i>Cantanhez</i>	<i>Xitole</i>	<i>0.69</i>	<i>0.31</i>
t15	0.0	14.7	1.9	Cufada	Xitole	0.21	0.79
t16	3.5	39.5	43.9	Boé	unknown	0.15	0.85
t26	<i>0.0</i>	<i>0.0</i>	<i>0.0</i>	<i>Cufada</i>	<i>unknown</i>	<i>0.35</i>	<i>0.65</i>
t41	11.1	40.1	0.0	Cufada	Cacine	0.22	0.78
t43	6.5	32.5	9.5	Cufada	Cacine	0.25	0.75
t54	0.0	5.1	1.38	Cufada	Cacine	0.24	0.76
t25	<i>0.0</i>	<i>0.0</i>	<i>0.0</i>	<i>Boé</i>	<i>unknown</i>	<i>0.05</i>	<i>0.95</i>
t55	1.33	18.4	0.0	Cufada	Buba	0.68	0.32

4.5 – Discussion

4.5.1 - Amplification success and genotype reliability

Even though the faecal samples were relatively fresh and collected minutes after detection of groups, only 45.4 % of the extracted samples could be included in the final dataset. Most of the samples were excluded due to inconsistent amplification of nuclear DNA. Since we found great variation in the reliability of genotypes in samples collected on

the same day, DNA degradation do not seem to be the cause of the low success rate. Instead, the presence of PCR inhibitors (derived from the diet), which were not eliminated during the DNA extraction process (Beja-Pereira *et al.*, 2009), seems more likely. Nevertheless, the selection done at the early phases of the genotyping process provided the final dataset with samples with a high amplification success across loci (80.8 %) and reliable genotypes (average QI of 0.83). The final ADO and FA rates (11 to 32 % and 1 to 5 %, respectively) were in the range found in other primate studies, either using similar extraction protocols (for example 4-32% ADO and 1.8-9.9% FA, Fickenschner 2010) or more recent developed extraction methods (5 to 39% ADO, Quéméré *et al.*, 2010).

4.5.2 - Genetic Diversity

The genetic diversity, derived from microsatellite data estimated for the southern Guinea-Bissau baboons (mean number of alleles = 4.24; U_{He} = 0.58) corresponds to the estimated values for Guinea baboons from a neighbouring population in Senegal (He = 0.59; Fickenschner, 2010). Both Guinea baboon populations seem to have lower genetic diversity compared to baboon subspecies (Yellow baboons: He = 0.73-0.79, St. George *et al.*, 1997, He = 0.65-0.80, Burrell, 2008, He = 0.67, Storz *et al.*, 2002; Gray-footed baboons: He = 0.56-0.72, Kinda baboons: He = 0.75, Burrell, 2008). This pattern is also present when comparing Guinea-Bissau baboon's mtDNA data (π = 1.3%±0.0011) with of other subspecies (gray-footed π = 5.3%, yellow π = 8.6% and kinda π =3.6%, Burrell, 2008). Low genetic diversity for both nuclear and mtDNA may suggest a prolonged bottleneck for Guinea baboon (Gaines *et al.*, 1997; Wilson *et al.*, 1985). Low diversity might be related to a founder effect due to the relatively fast colonisation of the West Africa by the subspecies (Zinner *et al.*, 2009). In fact, we found that the most common haplotypes exhibited a star-shape phylogeny, suggesting a population expansion after a bottleneck event (Slatkin and Hudson, 1991). However, comparing results between studies using different genetic markers should be done with caution due to the ascertainment bias caused by the use of very variable loci, which could lead to false inferences about past demographic changes (Amos and Harwood, 1998).

Boé possessed the highest genetic diversity among all sampling locations and is relatively centrally located in the Guinea baboons' distribution, when compared with Cufada and Cantanhez. Therefore, a greater number of dispersing individuals may be able to arrive at this population (Eckert *et al.*, 2008). Boé was the most differentiated area at both

nuclear and mtDNA level and showed positive F_{is} values. However, these results might not necessarily imply inbreeding due to isolation, as is often the inference. Apart from the Wahlund effect (heterozygosity deficit due to sampling across multiple populations), non-random mating due to social structure (mating among kin) can increase observed homozygosity in relation to expected homozygosity levels in a population in HW equilibrium (Di Fiore, 2003). In the Boé area, samples from only three social units in close geographic proximity were collected whereas, in the Cantanhez and Cufada regions, a higher number of social units over a broader geographic scale were sampled. Therefore, the effect of the social structure could be less pervasive in the Cantanhez and Cufada data.

4.5.3 - Population Structure

The genetic structure inferred by Bayesian clustering using microsatellite loci was relatively concordant between approaches and highlights the most dominant pattern of genetic structure within Guinea-Bissau as two moderately differentiated genetic units ($F_{st} = 0.087$), with slightly different levels of genetic diversity ($H_{cluster1} = 0.51 \pm 0.14$ and $H_{cluster2} = 0.59 \pm 0.08$, respectively). Non-spatial BAPS clustering of individuals and GENELAND (correlated alleles frequencies), however, hint at a higher number of genetic units within our dataset. When compared to STRUCTURE, these models have previously been described to overestimate population structure in cases of low population differentiation (Guillot *et al.*, 2005a; Latch *et al.*, 2006). The results of non-spatial BAPS were inconsistent clustering solutions, which did not converge in the independent runs. The differences between the STRUCTURE and non-spatial BAPS algorithms might explain some of the discordance (e.g. Frantz *et al.*, 2009) although in our study, the underlying social structure might have also influenced the results. The related individuals within each social unit could present similar alleles frequencies and, consequently, be clustered together by non-spatial BAPS as population units, increasing the number of clusters. Anderson and Dunham (2008) showed that the delimitation of genetic units as inferred by STRUCTURE can be influenced by the signal of the social structure. The non-spatial BAPS results could be influenced in a similar fashion. The inclusion of geographic location as a prior in non-spatial BAPS led to convergence with the STRUCTURE clustering solutions, which was already found by other authors (Frantz *et al.*, 2006). GENELAND using the correlated allele frequencies model grouped the samples in five clusters and the genetic discontinuities found in this analysis were relatively concordant with the spatial interpolation. Such a result highlights the co-existence of genetically divergent individuals in the same social unit (for

example in the Quebo-Sutuba social unit in Cantanhez), indicating that the extra genetic clusters might have some biological relevance. Nevertheless, the use of the different independent methods pointed to the same conclusion, which increases confidence in the results discussed below.

The high proportion of admixed individuals suggests some form of contact between sampling locations. This was not in agreement with our expectations of isolation between sampling locations but also not unexpected when considering the apparently high mobility of Guinea baboons (40Km per day, Galat-Luong personal communication) and the dimension of their home ranges (25 Km², Senegal, Fickenscher, 2010). Samples with distances of up to 33 Km between them were genetically similar, which could be attributed to the sampling of related individuals of the same family group at shorter distances (Peakall *et al.*, 2003).

The first distance class of genetic dissimilarity (66 Km) encountered is within the range of maximum dispersal distances estimated in other baboon subspecies. Yellow baboons disperse to social groups up to 15-22 Km away (St.George *et al.*, 1996) or between 11-19.1 Km (Tung *et al.*, 2008), although sometimes reaching distances of 30 Km (Alberts and Altmann unpublished data, cited in Tung *et al.*, 2008). Hamadryas baboons tend to disperse preferentially to closely *One Male Units* (OMUs) (Hammond *et al.*, 2006) and in Senegal, the Guinea baboons seem to follow this pattern, with significant genetic differentiation being reached at distances of 50 Km (Fickenscher, 2010).

Three of the results obtained in this study were not predicted. First, in comparison with Senegalese baboons, the Guinea-Bissau population seems to be less structured. Using microsatellite data, Fickenscher (2010) found three genetic clusters within a linear distance of 66 Km.

Second, the genetic similarity between samples distanced at 115.5 Km apart, as estimated using microsatellite data, was also unanticipated. This is related to the presence of migrants in the Cufada region, originally from the Boé region or from a genetically related population. Four first generation migrants were identified and, although we did not sample the original population, one individual was in fact assigned to the Boé region. Such a result can be explained by dispersal over long distances or by secondary dispersal, not yet described for the Guinea baboon subspecies (since the population structure was only studied in a maximum of 66 Km, Fickenscher, 2010). This pattern of dispersal has been observed in

Yellow baboons (Alberts and Altmann, 1995) and in Hamadryas baboons (Hammond *et al.*, 2006). However, we cannot exclude the hypothesis of migration from a population located on the northern side of the Corubal River, which might be genetically different from Cufada. Baboons are good swimmers (Smithers, 1959) and they are able to swim across watercourses if they feel threatened by predators (Busse, 1980). On the northern side of the Corubal River, hunting seems to be quite frequent as the putative origin of several bushmeat tissue samples was located here (Cossé and Xitole). Since baboons could potentially cross the river in Saltinho village (where it is only 130m wide), and the bushmeat samples collected are genetically close to the Boé population, it is plausible that the migrants sampled in Cufada could have dispersed from the northern part of the river.

Third, the proportion of admixed individuals varied between sampling regions. The Cantanhez and Boé regions showed lower proportions of admixed individuals whereas the Cufada region had the highest proportion. According to these results, Cufada can be considered a contact zone between populations. In Cufada, genetically divergent individuals co-exist in the same social unit ($q > 0.80$ to each cluster) and the proportion of admixed individuals was the highest (53% of individuals). Asymmetric gene flow to Cufada from Boé or from a genetically closely related population, along with the high percentage of admixed individuals suggests that this area comprises a demographic sink (Howe and Davis, 1991).

The higher preference of immigrants to Cufada and not Cantanhez is probably not related to habitat, although differences do exist. While mosaics of dry forest-savannah woodland predominantly characterise Cufada, Cantanhez is mainly comprised of patches of Guinean forest (Catarino *et al.*, 2001). However, Guinea baboons can persist equally well in either of these habitats as this subspecies is distributed along a gradient of ecosystems, ranging from sub-humid forests in the south to desert in the north of the distribution (Galat-Luong *et al.*, 2006). The large geographic distance between Boé and the other regions alone also does not seem to explain why Cufada receives more immigrants than Cantanhez. At least two social units in Cantanhez (Porto Gandamael – 119 Km and Quebo-Sutuba – 123.80 Km) share similar distances to Boé with the social units in the Cufada region where migrants were sampled (Sr. Soares 1 and Bakar Contê, 120 Km and 100 Km, respectively). Second, the Cantanhez social units are genetically more similar to the Cufada social units at distances of 51 Km (for example Botchê Cule and Guebombol) but are genetically more different from other social groups distancing only 39 Km (Botchê Cule and Bakar Contê).

MtDNA inferred population structure was very similar to that found in a Eritrean population of Hamadryas baboons (Hapke *et al.*, 2001): the distribution of the haplotypes was not related to the sampling locations, no significant isolation by distance was found, a greater percentage of total variation was present within social units and, finally, there were haplotypes shared by individuals separated from each other by more than 50 Km. We found that in Guinea-Bissau, geographically close individuals (e.g. 16.5 Km) were different, with significant genetic similarity occurring at a distance of 148 Km. Such results suggest historical female-biased dispersal. This pattern is in sharp contrast to Burrell's (2008) findings for Yellow and Gray-footed chacma baboons, two subspecies described to have female philopatry and male dispersal. Burrell (2008) showed that most of the mtDNA variation is present among groups (76 % - 100 %) and also describes social groups where only one haplotype was found.

Nuclear variation in Guinea baboons from Guinea-Bissau seems to be structured similarly to mtDNA variation: we found that 95.1 % of the variance was present within social units while only 1.6 % of variation was present among social units. This is not in disagreement with female-biased dispersal (Hammond *et al.*, 2006; Melnick and Hoelzer, 1992) and corroborates the evidence for male philopatry found in the Guinea baboons from a Senegalese population (Fickenschner, 2010). A scenario where both sexes disperse could also explain the lack of structure for both maternally and bi-parentally inherited genetic markers (Avice, 2004; Hammond *et al.*, 2006).

Population structure inferred by mtDNA corroborates the pattern yielded by nuclear DNA of contact between sampling locations. However, the detection of genetic differentiation between regions seems to suggest that either historical or more recent gene flow have not been high enough to homogenise allele frequencies. Although distance seem to be the most likely explanation, we found a weak (although significant) linear correlation between genetic and geographic distances ($R^2 = 0.14$) when using microsatellite loci and no significant correlation when using mtDNA, when all samples were pooled or even in when pairwise comparisons of sampling locations were made.

After removing the four migrants identified from the Cufada region in the microsatellite dataset and repeating the Mantel test with all samples, the linear correlation between geographic and genetic distance became stronger ($R_{xy} = 0.4$, $p < 0.001$, $R^2 = 0.16$) suggesting that the presence of these migrants in the Cufada region masked the signal for a

pattern of isolation-by-distance. For mtDNA, the differentiation between sampling regions are most certainly related to differences in the frequencies of haplotypes grouped in Cluster 3, 4 and 6, which occurs more frequently in the Boé region but not so commonly in the Cufada and Cantanhez regions.

4.5.3.1 - Factors affecting Population Structure

Hunting pressure, as initially expected, does not seem to constitute a barrier to dispersal. The genetic discontinuities identified, using both microsatellite loci and mtDNA, were not concordant with the geographic location of human settlements but were caused by the presence of genetically differentiated individuals co-existing in the social units. Furthermore, the similarity between the interpolation analyses using the different genetic markers pointed to a recent contact between genetically differentiated individuals. The social units grouped on either side of the genetic discontinuity (Sr. Soares 1, Bakar Contê and Quebo-Sutuba) share individuals genetically differentiated at both the nuclear and mtDNA level. The pattern found in this study is remarkably similar to what is observed in hunted populations of other species (for example Jedrzejewski *et al.*, 2005; Robinson *et al.*, 2008; Gobush *et al.*, 2009).

Two different scenarios can potentially explain the patterns observed in the Guinea-Bissau baboon population. First, the dispersal distances for baboons within Guinea-Bissau seem to have been increased. This can be justified as a means to occupy empty areas (Ji *et al.*, 2001; Perrin and Goudet, 2001). Cufada is very closely located to the main road and hunting within the park is now forbidden. However, before the establishment of the park in 1999 hunting activities were common in this area (Casanova and Sousa, 2007; Chapter 3; Ferreira, 1948), which probably led to a decrease in baboon numbers. As hunting pressure decreased inside the park, the baboons might have migrated towards the Cufada region to occupy the baboon-depleted habitat. Alternatively increased hunting pressure in areas between the sampled regions could have caused baboons to seek refuge in protected areas. This would also explain the genetic discontinuity found at entrance to the Cantanhez Peninsula (Quebo-Sutuba social unit). The pattern of recent contact is not so noticeable in Cantanhez, as it is in Cufada, probably due to difficulty for baboons in penetrating into this already habitat-fragmented Peninsula. This can be either due to a higher density of baboons inside the park or due to lower availability of space for baboons caused by a higher density of human population within this Park.

In this work we have shown that hunting practices can affect the population structure of a primate species. The Guinea baboon's ability to move over large distances prevented isolation of subpopulation but hunting practices seem to have induced a demographic sink. The effect of hunting pressure on dispersal behaviours should therefore be taken into consideration when investigating the genetic patterns of other primate species.

4.6 - References

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**Chapter 5 Does hunting pressure influence sex-biased
dispersal patterns? A comparative study in Guinea
baboons (*Papio hamadryas papio*)**

Amizade é paz

(Friendship is peace – by Zé Manel, Guinean-Bissau singer, exiled in the USA)

Does hunting pressure influence sex-biased dispersal patterns? A comparative study in Guinea baboons (*Papio hamadryas papio*)

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5.1 - Abstract

Anthropogenic hunting practices may induce a change in dispersal behaviour via group density variation across space or by inducing defensive behavioural responses. Here, we compared two populations of Guinea baboons (*Papio hamadryas papio*) subject to different levels of human pressures to test for changes in the composition of social units and in the dispersal behaviour. In Guinea-Bissau (GB), baboons have been heavily hunted and suffered a range contraction. In Senegal (SEN) baboons have increased in numbers and harvesting is not significantly affecting the population. By using a molecular sex determination protocol and thirteen microsatellite loci, we investigated differences in the proportion of males and females and the mean pairwise relatedness within social units. Furthermore, we compared sex-specific patterns of gene flow. We found in the anthropogenic-impacted population a pattern of lower ratio of males within social units and social units with un-related individuals. The clear female-biased dispersal pattern displayed in Senegal was attenuated in GB, where, in the same geographical scale, gene flow was mediated through by both sexes. Within GB, the origin of dispersing males, when compared with females, was predominantly from a genetically differentiated population, resulting in the formation of a contact zone. Results are discussed within the context of conditional-dependent dispersal strategies. For SEN males, philopatry could be a means to avoid competition with conspecifics and aggressive encounters while in GB male dispersal could result from higher hunting-mortality risk or as a means to increase reproductive outcome.

Keywords: Hunting, guinea baboons, sex-biased dispersal, condition-dependent dispersal

5.2 - Introduction

The sex-biased dispersal patterns in phylogenetic close-related species or populations of the same species are usually well conserved (Lawson Handley and Perrin, 2007; Strier, 2007). During the species evolutionary history, sex-specific selective forces encouraging or opposing to dispersal have shaped the patterns displayed (reviewed in Lawson Handley and Perrin, 2007). When the benefits of moving from the natal to the breeding group surpass the costs of this behaviour, dispersal presents a selective advantage (Bowen Jones and Pendry, 1999; Ronce, 2007; Clobert *et al.*, 2009). Selection imposed on individual dispersing behaviours can influence population dynamics, either locally or in a meta-population scale (Ronce, 2007; Clobert *et al.*, 2009; Hawkes, 2009).

Dispersal behaviours are also context-dependent (Bowler and Benton, 2005; Ronce, 2007; Clobert *et al.*, 2009). The preference to disperse depends on ecological, demographic and social cues received during the individual's lifetime (Bowler and Benton, 2005; Isbell and Van Duren, 1996; Strier, 2007). Density, interactions with conspecifics or with other species, sex ratio, relatedness among neighbouring groups and intrinsic patch characteristics (available food, size and isolation) seem to greatly influence both dispersal rates and distances (Bowler and Benton, 2005; Clutton Brock and Lukas, 2012).

In primate species, dispersing behaviours can include movements to an unfamiliar area, movements within the natal range or both (Isbell and Vuren, 1996). In general, the suggested benefits for social dispersal encompasses the avoidance of kin competition, aggression and inbreeding (Lawson Handley and Perrin, 2007; Clutton Brock and Lukas, 2012). Eviction, threatening factors affecting dependent individuals (predators or infanticidal males) and abduction by males (e.g. hamadryas baboons) have been shown to induce female social dispersal (Lawson Handley and Perrin, 2007; Jack and Fedigan, 2008; Clutton Brock and Lukas, 2012).

Dispersal of individuals to a different social group can lead to aggression from conspecifics (Isbell and Vuren, 1996; Lawson Handley and Perrin, 2007; Clutton Brock and Lukas, 2012). Female social dispersal in particular is frequently limited by the degree of overlap of a group's home range with others (Isbell and Van Duren, 1996). For females,

hindering transfer between groups can decrease survival rate and breeding success (Clutton Brock and Lukas, 2012) because female reproduction is more dependent on food resources (Isbell and Van Duren, 1996; Altmann, 1990). On the other hand, the decrease in foraging efficiency and elevated mortality risk increase the costs involved in movement to unfamiliar areas (Waser *et al.*, 1994; Isbell and Vuren, 1996; Lawson Handley and Perrin, 2007; Clutton Brock and Lukas, 2012). Mortality can be sex-specific (Waser *et al.*, 1994), arise from higher energetic costs (Johnson *et al.*, 2009) or from a greater exposure to predation (Alberts and Altmann, 1995; Clutton Brock and Lukas, 2012) but is sometimes anthropogenic-related (Ellsworth *et al.*, 1994; Bonnet *et al.*, 1999).

Anthropogenic hunting, one of the main threats affecting primate species today (Mittermeier, 1987; Chapman and Peres, 2001; Di Fiore, 2004; Chapman *et al.*, 2006), usually impacts species differently (Fa and Brown, 2009). Within species, hunting can either be non-selective or directed to a specific age-class or sex (Greene *et al.*, 1998). While primate males are frequently targeted for greater economic return (Mittermeier, 1987) they are also more conspicuous within groups (Cowlshaw and Dunbar, 2000; Fa and Brown, 2009), for example, when vocalizing during attacks (Fischer *et al.*, 2001). When trading of juveniles is profitable, lactating females are also targeted (Mittermeier, 1987; Starin, 1989; Ceballos-Mago *et al.*, 2010).

By modifying species behaviour, age-classes or sex ratio (Cowlshaw and Dunbar, 2000; Loveridge *et al.*, 2007) or the number or density of groups (Watanabe, 1981; Rosenbaum *et al.*, 1998; Nijman, 2004; Allendorf *et al.*, 2008; Kuehl *et al.*, 2009), hunting pressure can potentially disturb primate population structure (Harris *et al.*, 2002; Allendorf *et al.*, 2008), although the effects are not well understood in those species.

As a result of multiple effects, effective dispersal between subpopulations or social groups can be greatly affected (Allendorf and Hard, 2009). Gene-flow may be reduced if hunters target the dispersing sex (Ellsworth *et al.*, 1994) or if human settlements alter migration routes (Allendorf and Hard, 2009) or constitute significant barriers to dispersal (Liu *et al.*, 2008; Murtskhvaladze *et al.*, 2010). Additionally, density declines as a result of localized pressure can promote immigration (Matthysen, 2005; Allendorf *et al.*, 2008; Harris *et al.*, 2002; Delibes *et al.*, 2001). In such cases, hunted areas represent demographic sinks e.g. sites where deaths exceed births (Novaro *et al.*, 2000) and their existence diminishes the overall population size (Coltman, 2008).

Additionally, hunting practices can alter behavioural patterns. In an environment of high hunting pressure, some species may become more secretive, decreasing their vocalization rate (Croes *et al.*, 2006) or changing ranging patterns to avoid hunters (Tutin and White, 1999). Group size in social species can be enlarged by a defensive response to predation (Cowlishaw and Dunbar, 2000, but see Croes *et al.*, 2006), due to associations among social groups of different species (Noe and Bshary, 1997; Teelen, 2007; Oliveira and Dietz, 2011) or, sometimes, by merging with un-related conspecifics (Nyakaana *et al.*, 2001; Jedrzejewski *et al.*, 2005; Gobush *et al.*, 2009). In this cases, the population social structure can become stressed, with various consequences to the population growth (Archie and Chiyo, 2012).

Primate populations may respond to anthropogenic environmental changes by altering the dispersal rates of individuals usually remaining philopatric (Isbell and Vuren, 1996; Sugiyama, 1999). As a result, the species-specific dispersal patterns, usually biased towards one sex, can be reversed or become less intense (Strier, 2007; Pérez-Espona *et al.*, 2010). In primates, exceptions to the species normative pattern have been observed in cases of unusually high group isolation (e.g. *Pan troglodytes verus*, Sugiyama 1999) and when natural predators are absent (e.g. *Papio hamadryas ursinus*, Anderson pers. comm. in Isbell and Van Duren, 1996) but also under the influence of human's activities (e.g. *Macaca cyclopis*, Hsu and Lin 2001) and in areas of anthropogenic-hunting practices (Di Fiore *et al.*, 2009).

The aim of this study was to compare two Guinea baboon (*Papio hamadryas papio*) populations suffering from contrasting levels of human pressure. In Guinea-Bissau (GB), an apparently high hunting pressure has contracted the baboon range and population size during the last thirty years (Gippoliti and Dell'Omo, 2003; Casanova and Sousa, 2007; Cá, 2008; Costa, 2010). The high profitability of male baboon meat compared with other smaller bodied species (Chapter 3), along with the geographically widespread observation of baboons being kept as pets (Casanova and Sousa, 2007; Hockings and Sousa, 2011), suggests mortality of both lactating females and adult males.

In contrast, the Niokolo Koba National Park population in Senegal (SEN) is not greatly affected by harvesting (Galat-Luong *et al.*, 2006; Fickenschner, personal observation), although predation by lions (*Panthera leo*) seems to be significant (Galat-Luong *et al.*, 2006). Nevertheless, the population has increased in size, reaching

approximately 250,000 individuals in 1998 (Galat-Luong *et al.*, 2006). Recently, genetic evidence of female-biased dispersal and male philopatry was found (Fickenscher, 2010). The conclusions were in agreement with previous behavioural studies describing a high degree of tolerance and affiliative behaviours between adult males (Boese, 1973; Sharman, 1981; Galat-Luong *et al.*, 2006).

By using a molecular sex determination and microsatellite markers, we compared the GB and SEN populations focusing on: i) the number of males and females within social units, ii) the relatedness between individuals within social units and iii) sex-biased dispersal patterns (Prugnolle and de Meeus, 2007). The amalgamation of unrelated conspecifics in social groups is a possible hunting-driven behavioural response (Gobush *et al.*, 2009) so we expected to find lower levels of relatedness within GB social units. Sampling in GB was conducted at three geographically distinct sites, surrounded by areas where baboons persist in low densities or have recently disappeared. Considering the combined effects of lower groups density and higher perceived predation risk in GB, we expected to find a disrupted pattern of sex-biased gene flow when compared with SEN. We also assessed the proportion of males and females being traded at the GB bushmeat markets, by identifying the sex of baboon carcasses collected as part of additional work (Chapter 3).

5.3 - Methods

5.3.1 - Study sites and DNA Sampling

The faecal samples were collected in two locations roughly 159Km apart: Southern Senegal (SEN) in *Parc National du Niokolo Koba* (PNNK) and southern Guinea-Bissau (GB). Within PNNK, samples were collected in five locations, to a linear maximum distance of 70Km: *Gue Damantan* (GD), *Simenti* (Si), *Camp du Lion* (CL), *Lingue Kountou* (LK) and *Niokolo Koba* (NK). In southern Guinea-Bissau, samples were collected in three locations: Cantanhez Woodlands National Park (*Parque Nacional das Matas de Cantanhez*), Cufada Lagoons Natural Park (*Parque Natural das Lagoas da Cufada*) and in Boé region. Located in the southwest part of GB, Cantanhez and Cufada are separated by 40 Km. Boé region is separated roughly by 100Km to Cantanhez and Cufada and located in the southeast of this country (Fig. 5.1).

The sampling strategy applied was different between SEN and GB. In GB, sampling focused on foraging parties (with the exception of one social unit, sampled at a sleeping site) representing groups not larger than 50 individuals. The home range of social groups within localities was unknown at the beginning of the study. To ensure sampling of independent social units, each site was visited and sampled only once and wherever possible, sampling at different sites was conducted on the same day. Parks guards and guides aided sample collection. In Senegal, most samples came from the Si group, a troop under study by the Cognitive Ethology Laboratory of the German Primate Center (DPZ). SEN samples were either collected in sleeping sites or on the baboon's daily paths and locations were visited more than once.

Up to 5 ml faecal material, scraped from the exterior surface, was preserved using the two-step method (Roeder *et al.*, 2004), using 99% ANALAR ethanol and silica gel (GB samples: Type III, S-7625, Sigma-Aldrich® Company Ltd, Dorset, UK; SEN samples: orange silica beads, Roth, Kalsruhe, Germany). Samples collected in Senegal were exposed to an air-drying step before desiccation by silica beads.

The fourteen tissue samples collected in the bushmeat market in the capital of Guinea-Bissau (see Chapter 3) were preserved in 99% ANALAR ethanol until DNA extraction.

5.3.2 - Laboratory methods

The faecal samples collected in GB were extracted and genotyped in the laboratorial facilities of School of Biosciences, Cardiff University, UK while samples collected in Senegal were analysed in the laboratorial facilities in German Primate Center, Gottingen, Germany.

5.3.2.1 - DNA extraction

Total genomic DNA from faecal samples was extracted using the QIAamp®DNA Stool Mini Kit (QIAGEN ®) with slight modifications from the manufacturer's protocol (see Chapter 2 and Fickenschner, 2010 for more details). DNA extracts were eluted in 200ul Buffer AE for GB samples and eluted in 50ul HPLC water aliquots for SEN. After extraction, samples were stored at -20°C. Negative controls were subjected to all extraction procedures (and included in the following PCRs). Whole genomic DNA from bushmeat

tissue samples was extracted using DNeasy Blood and Tissue kits (Qiagen ©) (Chapters 2 and 3).

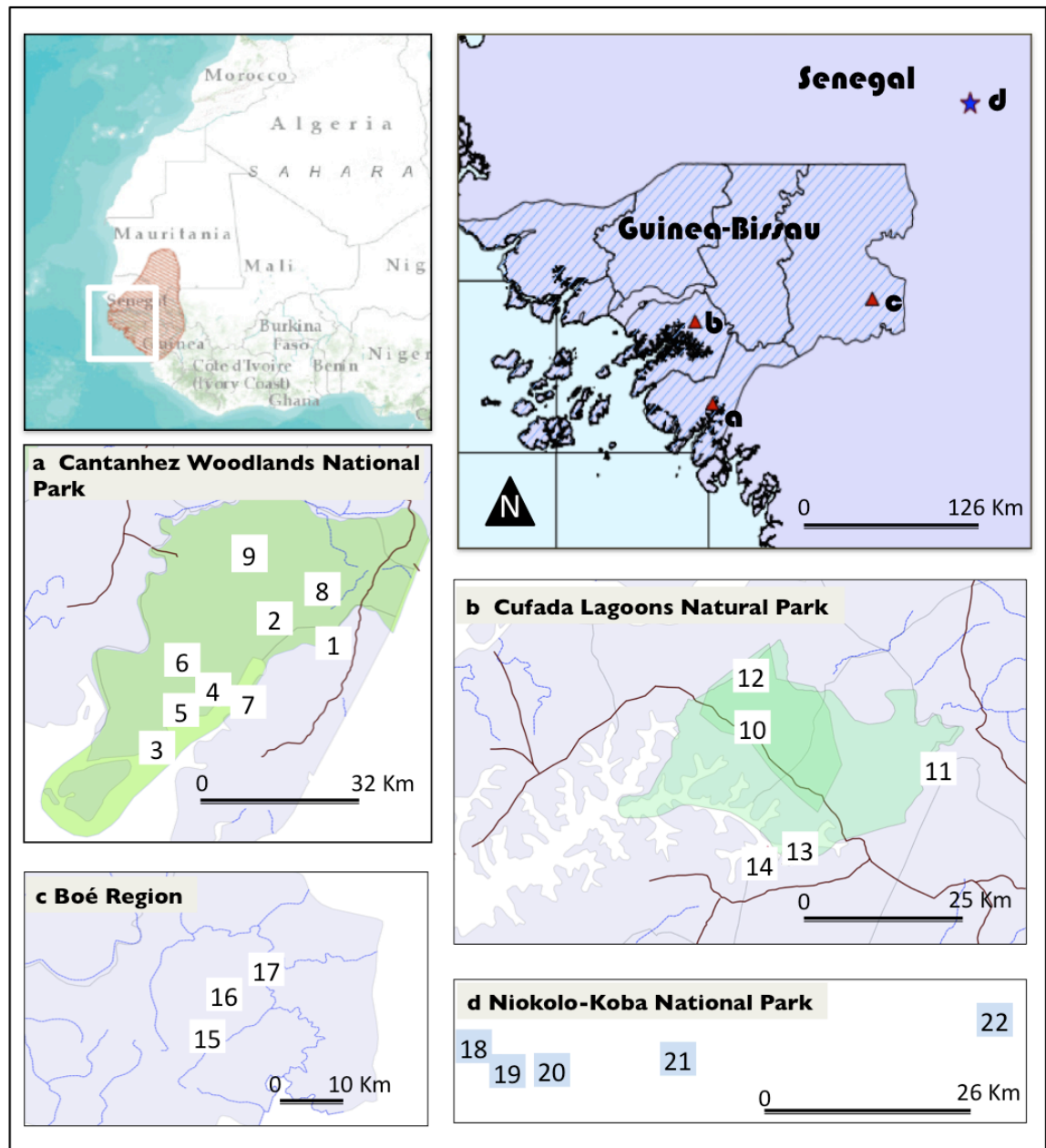


Figure 5.1: Sampling locations within Guinea baboon distribution range. Top left map show the Guinea baboon range, which was partially adapted from IUCN red list site (www.iucn.org). Top right map show the location of sampling sites within Guinea-Bissau (a – Cantanhez Woodlands National Park, b – Cufada Lagoons Natural Park, c – Boé region) and Senegal (d – Niokolo-Koba National Park). Numbers indicate location of sampled social units (see sampling effort in Table 5.1).

Table 5.1: Sampling effort in Guinea-Bissau (GB) and in Senegal (SEN) by sampling regions within each country and social units (within regions in GB). ID show the correspondence with the geographic locations of social units in Fig. 5.1. It is also indicated the name of social units and number of males (M) and females (F) included in the dataset (ND – no data obtained for sex determination protocol)

ID	Country	Region	Social Unit	M	F	ND	Total
1	GB	Cantanhez	Porto Gadamael	6	7	0	13
2			Amindara	2	5	0	7
3			Cabedu	3	5	1	9
4			Catomboi	3	7	0	10
5			Canamina	2	8	0	10
6			Caiquene	1	2	1	4
7			Cambeque	2	4	0	6
8			Quebo-Sutuba	1	3	0	4
9			Botchê-Culê	4	6	0	10
10		Cufada	Bubatchingue	8	13	0	21
11			Bakar Contê	4	6	1	11
12			Guebombol	1	2	2	5
13			Sr. Soares 1	3	4	0	7
14			Sr. Soares 2	1	9	0	10
15		Boé	Boé Belí	4	1	1	6
16			Boé Aicum	7	4	0	11
17			Boé Aicum Montanha	2	3	0	5
Total GB				54	89	6	149
18	SEN	PNNK	Gue Damantan (GD)	6	5	0	11
19			Simenti (SI)	66	42	0	108
20			Camp du Lion (CL)	5	6	0	11
21			Lingue Kountou (LK)	5	8	0	13
22			Niokolo Koba (NK)	15	7	0	22
Total SEN				97	68	0	165

5.3.2.2 - Microsatellite amplification

Thirteen microsatellite loci, with allele range size varying between 125 base pairs and 212 base pairs (bp), human-derived and with known cross-amplification in *Papio* (Bayes *et al.*, 2000), were amplified using five multiplex PCR systems. All markers were tetranucleotide repeats, with the exception of locus D7S503 (dinucleotide). DNA amplification was similar between studies, with minor differences. For SEN samples, locus D4S243 was co-amplified with locus D21S1442 (excluded from the analyses performed). For GB samples, locus D4S243 was amplified in a singleplex PCR (see Table 5.2 for details). All multiplexes were amplified in a 10ul volume using the QIAGEN Multiplex PCR Kit®, containing between 1.2ul (SEN) and 2uL (GB) of DNA extract. The final reaction concentrations consisted of 1x QIAGEN Multiplex PCR Master Mix®, 0.2uM of each multiplex primer mixture and 0.75uM BSA (Table 5.2). PCR cycling conditions started with a HotStarTaq DNA Polymerase activation step, during 15 min at 95°C, followed by denaturation step at 94°C for 30 sec, annealing step for 40 sec at between 50°C to 59°C (depending on the multiplex, Table 5.2) and extension at 72°C for 60 sec, repeated 40 cycles. The PCR ended with a final extension of 30 min at 72°C. PCRs were performed in an AB Applied Biosystems™ (California, USA) Veriti 96 Well Thermal Cycler for GB samples and in a Sensoquest labcycle for SEN samples. We avoided cross-contamination or contamination with external DNA by subjecting to UV lights irradiation all material used in the PCRs, by assemble the PCRs in a separated facility and by using sterile filter tips in all steps.

GB PCR products were run on an ABI 3730XL capillary analyser using MacroGen's Genescan service. M1, M3, M4 and M5 were analysed using a 16 GeneScan™ -500 LIZ® size-standard and M2 was analysed using 16 GeneScan™ -400 HD® size-standard. SEN PCR products were run on an ABI 3130XL Genetic Analyser (16 capillary sequencer, Applied Biosystems) using a 16 GeneScan™ -400 HD® size-standard.

Table 5.2: Multiplex composition (M1, M2, M3, M4) and singleplex PCR performed in this study and respective conditions. The loci identifier, GenBank code, final concentration and size range are also reported. The sex determination protocol (Sex_DET) was included in Multiplex 5.

MULTIPLEX	LOCUS	GENBANK CODE	CONCENTRATION (uM)	RANGE (bp)
M1 (57°C)	D13S765	G09003	0.15	200-212
	D12S375	G08936	0.1	164-184
	D3S1766	G08269	0.1	192-208
	D7S503	G18277	0.6	142-156
M2 (55°C)	D2S1326	G08136	0.3	192-208
	D14S306	G09055	0.2	161-181
	D1S533	G07788	0.4	187-203
M3 (59°C)	D8S1106	G09378	0.1	149-161
	D6S501	G08551	0.5	171-187
	D10S611	G08794	0.1	129-137
M4 (57°C)	D5S1457	G08431	0.1	125-137
	D7S2204	G08635	0.4	230-250
	D3S1768	G08287	0.1	193-212
M5 (58°C)	D21S1442	G08071	0.4	221-245
	Sex_DET	C. Roos	0.2	150 or 180
SINGLEPEX	D4S243	M87736	0.2	152-172

5.3.2.3 - *Genotyping Protocol*

The genotyping protocol followed a modified “multi-tubes” approach (Taberlet *et al.*, 1996). For GB samples, the number of repetitions across loci required to obtain 95% confidence in the genotypes was obtained using the software GEMINI, version 1.4.1 (GEMINI, Valière *et al.*, 2002). The allelic dropout (ADO) and false alleles rate (FA) of an initial dataset were estimated by a maximum likelihood approach (Johnson and Haydon, 2007a) in Pedant v1.0 (Johnson and Haydon, 2007b). Based in the results, four amplifications per locus per sample were performed and the consensus threshold estimated by GEMINI (Valière *et al.*, 2002) was used as a set of rules for the genotyping process. For SEN samples, four PCRs were attempted and alleles were accepted if observed at least twice. The reliability of genotypes was estimated using the “quality index” (QI) (Miquel *et al.*, 2006) and only the samples with a QI above 0.55 (GB) and 0.50 (SEN) were included in the datasets. Amplification success, ADO and FA were estimated according to Broquet and Petit (2004). Several precautions were taken to assure comparability of results: i) to control for the effect on allele sizes of the different fluorescent labels used (Sutton *et al.*, 2011), samples were exchanged and genotyped under both amplification protocols; ii) the

allele-calling method was combined between researchers; and iii) the QI, the amplification success, the ADO and the FA were estimated using the same protocol (Broquet and Petit, 2004) and compared.

Test for repeated individuals was performed using Excel microsatellite toolkit (Park, 2001) and GIMLET v1.3.3 (Valière, 2002), allowing for one mismatch or two, for samples being distinguished by only one locus with a homozygote genotype. The probability of identity (Waits *et al.*, 2001) was computed using GIMLET. CREATE 1.3 (Coombs *et al.*, 2008) was used to convert databases for statistical analysis.

5.3.2.4 - Molecular sex determination

Samples were sexed using a molecular approach designed by Christian Roos (unpublished). This protocol uses two primers, designed on the Dead Box gene (primer forward: GGACGRACTCTAGATCGGTA, primer reverse: GTNCAGATCTARGAGGAAGC). The primers amplified two fragments in males (length 150bp and 180bp) and only one fragment in females (length 180bp). For SEN samples, 5ul PCR product was visualized on 2% agarose gels (60 volt/hours) after a vacuum concentration step. To confirm accuracy, the PCRs were repeated 2 to 4 times. Sex was determined if the same result was observed unambiguously twice (males) or three times (females) from four repeats. For GB samples, the forward primer was end-labelled with a PETTM fluorescent dye (Applied Biosystems) and included in M5. The result was confirmed if observed at least three out of four repeats.

5.3.3 - Statistical analyses

5.3.3.1 - Males and Females

To determine possible differences between the number of males and females identified within SEN and GB social units, we estimated the proportion of females and males within each social unit and performed Mann–Whitney U tests to assess significance.

5.3.3.2 - Genetic Diversity

For comparison between GB and SEN datasets, the genetic diversity per locus was assessed. The Allelic Richness (AR), an allelic diversity estimation compensated for unequal sample sizes and the coefficient of inbreeding (Fis) was estimated using FSTAT

v2.9.3.2 (Goudet, 2002). Additionally, the observed (H_o) and expected (H_e) heterozygosity per locus and the test for deviations from Hardy Weinberg equilibrium were computed using GenAlEx version 6.3 (Peakall and Smouse, 2006). Linkage disequilibrium (LD) between all pairs of loci per sampling location was computed using FSTAT. We additionally estimated the genetic diversity for samples identified as males and females per sampling locations (Senegal, Cantanhez, Cufada and Boé). SEN samples were pooled since sampling was conducted in an almost continuous design. GenAlEx was used to estimate the number of alleles (N_a), number of effective alleles (N_e), H_o , H_e , Unbiased expected heterozygosity (UHe) and F_{is} .

5.3.3.3 - GB and SEN sex-biased dispersal patterns

Differences between males and females in instantaneous dispersal patterns can be detected using bi-parentally inherited markers, such as microsatellite loci (Goudet *et al.*, 2002; Prugnolle and de Meeus, 2007). The rationale behind these methods is that a sex-specific difference in the dispersal and consequent gene flow will affect the overall population structure. With time, individuals of the philopatric sex living in a social group will establish gene correlations. The dispersing individuals, on the other hand, will break down any gene correlations across groups. This will lead to similar allelic frequencies across subpopulations for the most frequently dispersing sex, while individuals of the philopatric sex will show higher population structure (Goudet *et al.*, 2002; Prugnolle and de Meeus, 2007).

The most commonly used methodologies include the comparison of relatedness and F_{is} between sexes within sub-populations and F_{st} among subpopulations (Goudet *et al.*, 2002). Other methods have also been used (Goudet *et al.*, 2002), namely the corrected Assignment Index (AI_c) (Favre *et al.*, 1997), e.g. the probability of a genotype assigned to the set in which it is included and in each sub-population, the mean Assignment Index (mAI) for each sex and the respective variance (vAI_c). In addition, dispersing and resident sexes will also differ in their spatial genetic autocorrelation (Peakall *et al.*, 2003; Banks and Peakall, 2012; e.g. Beck *et al.* 2008). As sex-biased patterns depend on the scale considered (Fontanillas *et al.*, 2004), to compare between GB and SEN we selected samples separated by a maximum of 66Km in GB ($N = 111$, 37 males and 74 females) to allow for a direct comparison with the SEN sampling scheme. The GB sub-dataset (referred to as “GB66”) included samples from Cufada and Cantanhez (with the exception

of Guebambol and Cabedu social units) and excluded all the samples from Boé social units.

5.3.3.3.1 - Population structure of Males and Females

The population structure of males and females within 66Km for both countries was investigated using STRUCTURE version 2.1 (Pritchard *et al.*, 2000). The analysis was performed using the admixture model with parameters set to default, assuming correlated allele frequencies. We inferred clusters (K) between one and 8. Each run was preceded by a burn-in of 100,000 steps followed by four MCMC runs of 1,000,000 iterations. To determine the optimal number of K, STRUCTURE HARVESTER version 6.8 (Earl and vonHoldt, 2011) was used to process STRUCTURE results. The most likely K was estimated using the highest estimated log-likelihood [$\ln P(X/K)$] and the *ad hoc* statistic ΔK developed by Evanno *et al.* (2005). The posterior probability of K was also calculated (Pritchard *et al.*, 2000).

5.3.3.3.2 - Spatial Autocorrelation of Males and Females

We compared similarity of individuals for both sexes at different distance classes using spatial autocorrelation analysis (Smouse and Peakall, 1999) performed in GenAlEx. This analysis estimates an autocorrelation coefficient r , which varies between -1 and 1 and measures the genetic similarity (if $r > 0$) or genetic dissimilarity ($r < 0$) between pairs of individuals separated in space by any distance. The significance of r was obtained by a permutation process, in which an upper and lower bounds of a 95% confidence interval was obtained. If the observed r lies outside this confidence interval, it is considered significant. Firstly, the global autocorrelation coefficient r was calculated for both males and females in GB66 and SEN datasets across ten distances classes. Furthermore, we compared the pattern displayed by the sexes within GB66 and SEN and between GB66 and SEN. For this comparison, only four distance classes were used due to the lack of pairwise comparisons within SEN and GB. The test of significant heterogeneity between the overall patterns displayed by males and females in SEN and GB66 datasets and between sites was computed using the advanced multiple populations option in GenAlEx. This analysis also tests for significant differences between populations for each distance class (Smouse *et al.*, 2008).

5.3.3.3.3 – Comparison between GB and SEN social units groups and dispersal patterns

For descriptive purposes, we estimated the mean pairwise relatedness of social units sampled in SEN and GB66 and additionally for males and females. The Queller and Goodnight (1989) estimator was computed using GenAlEx. A permutation process implemented in this analysis tested for significant mean pairwise relatedness (QGMr) within groups and the upper and lower bounds of the 95% confidence interval were obtained. The QGMr is considered significantly different from zero relatedness (e.g. mean pairwise un-relatedness) if it lies above the confidence interval. To estimate a 95% confidence around the QGMr relatedness estimator, 9,999 bootstraps were used. We also tested for differences in the average relatedness within social units. We assessed the differences in mean relatedness between SEN and GB66 social units and between sexes using FSTAT that uses a randomisation process to assess significance. In FSTAT, relatedness (*Relat*) is calculated using an estimator equivalent to Queller and Goodnight (1989) and corrected for inbreeding (*Relac*) (Goudet, 2002).

We also used FSTAT to test for differences in the social units from SEN and GB and different dispersal pattern between the sexes (Goudet *et al.*, 2002). Differences on *Fst*, *Fis*, *Ho*, within group gene diversity (*Hs*), *Relat* and *Relac* between sexes were estimated and tested by a randomisation method to assess significance. A two-sided test using 1,000 permutations was performed assuming that males were the most philopatric sex. This analysis was conducted for both GB66 and SEN datasets and separately for Cufada and Cantanhez. Samples were grouped according with the social unit where sampled. Among subpopulations, higher *Fst* values are expected for the philopatric sex. At the subpopulation level, due to the presence of pre-dispersal and immigrant individuals, the most frequent dispersing sex is expected to display a heterozygote deficit (the Wahlund effect) and therefore, higher and positive *Fis* values (Goudet *et al.*, 2002). *Ho* should vary between sexes if significant differences in inbreeding are found. Within social groups, lower levels of relatedness are also expected for the dispersal sex while lower levels of *Hs* are expected for the philopatric sex (Goudet *et al.*, 2002).

5.3.3.3.4 - *mAIC* and *vAIC*

The dispersing sex in each subpopulation is expected to show significant lower *mAIC* and higher *vAIC* values, when compared with the philopatric sex (Goudet *et al.*,

2002). mAIC and vAIC were estimated using GenAlEx. After estimating the individual AIC, this procedure tested the difference in the mAI distribution between males and females by a Mann Whitney U-test. In GenAlEx this analysis requires samples with no missing data. Therefore only 81 samples from GB66 (27 males and 54 females, 68 in Cantanhez and 13 in Cufada) and 151 samples from SEN (90 males and 61 females) were used. Tests were conducted for GB and SEN and separately for Cufada and Cantanhez social units included in GB66 dataset.

5.3.3.4 - GB sex-biased dispersal patterns

The population structure of males and females using all samples collected in GB was investigated using STRUCTURE version 2.1 (Pritchard *et al.*, 2000) with parameters as described in the previous sections. Additionally, we used BAPS spatial clustering of individuals (Corander *et al.*, 2006; Corander *et al.*, 2008a). In BAPS, we used priors for K of 10, 15 and 20, repeated 5 times. To assess repeatability, we performed five independent runs.

5.4 - Results

5.4.1 - DNA Extraction and Amplification

For GB study, 149 samples were included in the final dataset (QI > 0.55, averaging 0.87 mean across loci). For SEN study, 165 different individuals were included in the final dataset (QI > 0.5, mean 0.86 across loci). The probability of identity (pID) using this set of loci was of 2.20×10^{-10} and the probability of identity among sibs (pIDSib) was of 7.02×10^{-5} . The studies did not vary significantly in the QI of the samples included in the respective final datasets (Wilcoxon Signed-Rank test; $z = 0.96$, $p = 0.169$). However, amplification success across loci was significantly higher for SEN samples (97.44 %) when compared with GB samples (84.24 %) (Wilcoxon Signed-Rank test, $z = -3.16$, $p = 0.008$).

ADO did not vary significantly between studies (mean ADO GB = 14.17 % and mean ADO SEN = 15.66 %, Wilcoxon Signed-Rank test, $z = -0.65$, $p = 0.26$). Overall across loci, we found significant difference on FA rate between databases (2.49 % and 4.35 %, GB and SEN respectively, Wilcoxon Signed-Rank test, $z = -0.89$, $p = 0.0084$).

ADO and FA rate estimated for molecular determination protocol in GB dataset was of 9 % and 1%, respectively.

5.4.2 - Males and Females

The GB dataset comprised 55 males and 89 females. The sex of six samples remained undetermined. Out of the 14 samples collected at the bushmeat markets, 8 males and 6 females were distinguished. The SEN dataset included 97 males and 68 females.

The proportion of males and females identified within each social group showed significant variation, both across datasets and between regions within GB (Fig. 5.2). While within SEN social units, a greater proportion of males was identified (mean proportion males = 0.54; mean proportion females = 0.46, SD = 0.01), the opposite pattern was found in GB (mean proportion males = 0.38; mean proportion females = 0.62; SD = 0.08) (Fig. 5.2). This difference across SEN and GB was significant (Man Whitney U test, one tailed: $U = 68$, $p = 0.047$).

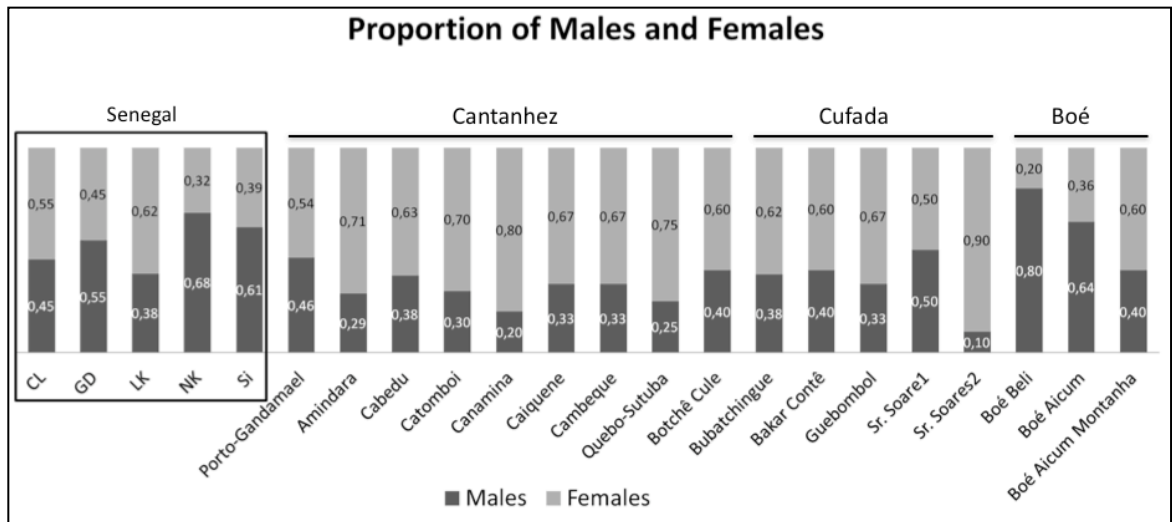


Figure 5.2: Proportion of Males and Females per social unit within each sampling location (Senegal, Cantanhez, Cufada and Boé).

Within GB, however, this pattern was only observed for Cantanhez and Cufada social units (Cantanhez mean proportion of males = 0.32; mean proportion females = 0.68, SD = 0.15; Cufada mean proportion males = 0.35; mean proportion of females = 0.65, SD = 0.15) (Fig. 5.2). The comparison between the proportion of males and females identified in Cantanhez + Cufada and SEN social units was highly significant (Man Whitney U test, one tailed: $U = 63$, $p = 0.004$). The social units sampled in Boé showed a pattern more comparable to the SEN social units, although with a greater variation due to a smaller

sample size (mean proportion of males = 0.61; mean proportion of females = 0.39, SD = 0.20) (Fig. 5.2).

5.4.3 - Genetic Diversity

The thirteen loci genotyped presented between three and seven alleles per locus (Table 5.3). The mean number of alleles for GB was 5.0 and was 5.3 for SEN. The values were relatively high for most loci (varying between 0.77 and 0.35). Mean H_e across loci (0.59) and AR (4.69 and 5.31, GB and SEN respectively) was very similar between datasets. Although GB baboons showed lower H_o (0.57) and positive F_{is} values (0.034) when compared with the Senegalese baboons (H_o = 0.62 and F_{is} = - 0.055), these differences were not significant. Overall, fewer loci were in Hardy Weinberg disequilibrium for GB than SEN (Table 5.3), which might be the result of the higher population structure in SEN.

Table 5.3: Genetic Diversity per locus for the overall datasets. AR (Allelic Richness), coefficient of inbreeding (F_{is}), observed and expected heterozygosity (H_o and H_e) is indicated per locus and across loci. Tests for Hardy Weinberg deviation (HW) are also referred. NS=not significant, * p <0.05, ** p <0.01, *** p <0.001

GB (N=149)	N	AR	F_{is}	H_o	H_e	HW
D12S375	147	6	0.051	0.57	0.60	*
D7S503	148	5	0.116	0.46	0.52	NS
D3S1766	149	5	0.080	0.61	0.66	NS
D13S765	138	4	-0.071	0.63	0.59	NS
D10S611	145	3	0.125	0.30	0.35	NS
D6S501	147	5	-0.030	0.68	0.66	NS
D8S1106	148	4	0.007	0.60	0.60	NS
D3S1768	149	5	-0.015	0.50	0.50	NS
D7S2204	142	5	-0.059	0.64	0.60	NS
D5S1457	126	4	0.043	0.51	0.53	NS
D4S243	147	5	-0.020	0.72	0.70	NS
D2S1326	126	4	0.134	0.48	0.55	*
D14S306	147	6	0.121	0.69	0.78	*
Overall	143 ± 2.26	4.69 ± 0.2	0.034 ± 0.02	0.57 ± 0.03	0.59 ± 0.03	

SEN (N=165)	N	AR	F_{is}	H_o	H_e	HW
D12S375	164	5	0.01	0.76	0.77	**
D7S503	161	7	-0.032	0.79	0.76	NS
D3S1766	165	3	-0.001	0.36	0.36	NS
D13S765	165	5	-0.02	0.50	0.49	NS
D10S611	163	4	-0.23	0.65	0.53	*
D6S501	165	5	-0.08	0.72	0.67	*
D8S1106	164	5	0.009	0.54	0.55	NS
D3S1768	165	5	-0.12	0.60	0.54	*
D7S2204	163	6	-0.002	0.74	0.74	NS
D5S1457	163	4	-0.12	0.50	0.43	NS
D4S243	165	7	-0.15	0.82	0.71	***
D2S1326	163	7	-0.08	0.60	0.55	NS
D14S306	165	6	0.07	0.54	0.58	NS
Overall	163.9 ± 0.4	5.31 ± 0.4	-0.055 ± 0.02	0.62 ± 0.04	0.59 ± 0.04	

Twenty-eight pairwise comparisons between loci displayed significant LD values (at $\alpha = 0.05$) for SEN, when the analysis was conducted for the whole dataset, which decreased to only four after applying Bonferroni correction for multiple comparisons (using $\alpha = 0.000321$). In contrast, for the GB dataset, only 12 pairs of loci showed significant LD values, at $\alpha = 0.05$. No pairs of loci showed LD after applying Bonferroni correction.

The genetic diversity in males and females did not vary substantially between sampling locations within SEN and GB, with the exception of males in Cantanhez and Boé (Table 5.4). Cantanhez males showed a slightly lower H_o , H_e and UHe compared with the remaining locations and Boé samples possessed a lower H_o . Overall when compared with SEN males, GB males displayed more positive F_{is} values, in particular Boé. This pattern was not observed for females (Table 5.4). With respect to number of effective alleles, H_o , H_e and UHe , females were similar across sampling locations.

Table 5.4: Genetic Diversity across loci for males and females divided by each sampling location. N_a (number of alleles), N_e (number of effective alleles), observed, expected and unbiased expected heterozygosity (H_o , H_e , UHe), coefficient of inbreeding (F_{is}) is indicated.

Locations	Males						
	N	N_a	N_e	H_o	H_e	UHe	F_{is}
Senegal	97	5.20 ± 0.30	2.70 ± 0.20	0.62 ± 0.04	0.59 ± 0.03	0.60 ± 0.03	-0.04 ± 0.03
Cantanhez	24	3.69 ± 0.21	2.20 ± 0.19	0.51 ± 0.04	0.51 ± 0.04	0.52 ± 0.04	0.00 ± 0.04
Cufada	17	4.15 ± 0.25	2.79 ± 0.16	0.59 ± 0.03	0.62 ± 0.02	0.64 ± 0.02	0.06 ± 0.03
Boé	13	4.08 ± 0.26	2.53 ± 0.16	0.48 ± 0.05	0.59 ± 0.02	0.62 ± 0.02	0.20 ± 0.07

Locations	Females						
	N	N_a	N_e	H_o	H_e	UHe	F_{is}
Senegal	68	4.77 ± 0.40	2.69 ± 0.30	0.63 ± 0.05	0.58 ± 0.04	0.59 ± 0.04	-0.08 ± 0.02
Cantanhez	47	4.10 ± 0.24	2.45 ± 0.24	0.57 ± 0.04	0.56 ± 0.03	0.56 ± 0.03	-0.03 ± 0.03
Cufada	34	4.08 ± 0.24	2.44 ± 0.18	0.62 ± 0.04	0.56 ± 0.03	0.57 ± 0.04	-0.12 ± 0.04
Boé	8	3.39 ± 0.29	2.64 ± 0.28	0.69 ± 0.06	0.57 ± 0.04	0.62 ± 0.04	-0.21 ± 0.08

5.4.4 – GB and SEN sex-biased dispersal patterns

5.4.4.1 - Population Structure between Males and Females

STRUCTURE analysis did not detect any structure for either males or females in GB66 (Fig. 5.3). The clustering solution with the largest Log (likelihood) and posterior probability across all runs was $K = 1$ (Posterior Probability $_{K1} = 0.99$). This analysis also failed to identify any structure for SEN females, contrary to SEN males (Posterior Probability $_{K1} = 0.99$). When using the ΔK method (Evanno *et al.*, 2005), $K = 2$ was the

highest modal value in the ΔK distribution (Fig. 5.3). Nevertheless, a second highest modal value appeared when $K = 4$, which is also the clustering solution with highest posterior probability (Posterior Probability_{K4} = 0.99) and with the largest Log (likelihood) (Fig. 5.3).

Based in the results given by the ΔK method for SEN males ($K = 2$), we followed the approach of Beaumont *et al* (2001) of plotting the ranked individual q values. This method intends to detect any break in the q values that would aid the assignment of individuals to the clusters. As the ranked individual q values were nearly continuous (not shown), we assigned individuals to each cluster when $q > 0,80$ and all other individuals were treated as a product of admixture between clusters. Overall, 93 samples were assigned to Cluster 1, 86 samples were assigned to Cluster 2 and 96 samples were considered admixed between clusters.

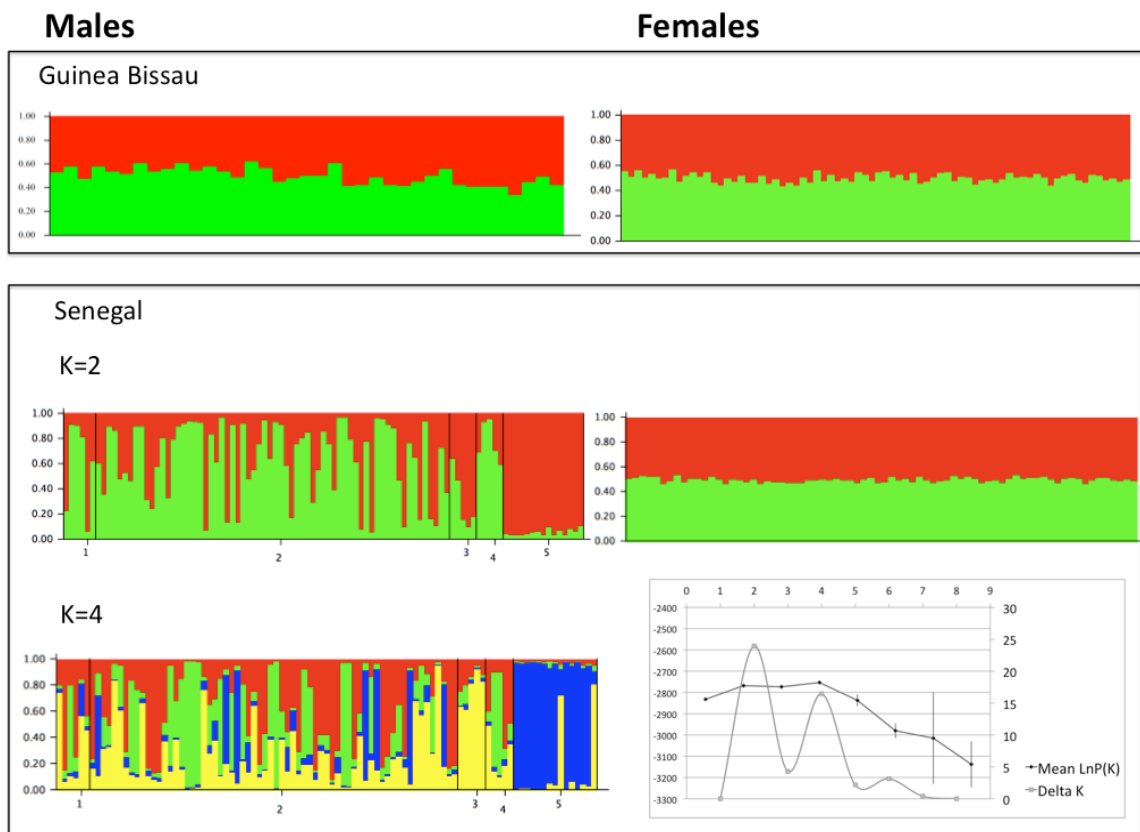


Figure 5.3: Results of the STRUCTURE analysis for GB and SEN females and males. In the graphs, a single vertical bar represents each baboon. The colours show the q membership of individuals in the two clusters. For SEN males, both $K=2$ and $K=4$ clustering solution are showed. The sampling location is indicated below STRUCTURE graphs: 1– GD, 2 – SI, 3 - CL, 4 – LK and 5 – NK. The graph on the bottom right show DELTA K and Mean Ln P(K) values as a function of number of putative populations (K).

The social units differed in the number males assigned to each of the clusters. In GD social unit (1 - GD, Fig. 5.3), 50% of individuals were assigned to Cluster 1. Only one individual was assigned to Cluster 2 and two individuals were considered admixed. In SI, 53% of the individuals were assigned to Cluster 1, 33% were considered admixed and 13.6% were assigned to Cluster 2 (2 - SI, Fig. 5.3). In the CL social unit, 80% of individuals were considered admixed and only one individual was assigned to Cluster 1 (3 - CL, Fig. 5.3). In the LK social unit, 60% of individuals were assigned to Cluster 1 and two individuals were considered admixed (4 - LK, Fig. 5.3). In contrast, 86% of the individuals sampled in NK were assigned to Cluster 2 and only two individuals were considered admixed (5 - NK, Fig. 5.3).

5.4.4.2 - Spatial Autocorrelation of Males and Females

Spatial autocorrelation analysis revealed several classes of significant positive and negative spatial genetic structure within GB66. For males in GB66, individuals were genetically similar at 19.8 Km and 26.4 Km. These distances represents pairwise comparisons between samples collected within Cantanhez and Cufada areas. In contrast, at larger distance classes (at 46.2 and 66 Km) individuals are significantly dissimilar. The pattern found for females in GB66 was quite similar. Females were significantly similar at shorter distances (6.6 and 13.2 Km) and dissimilar at larger distances (59.4 Km and 66 Km). Nevertheless, a class of significant positive spatial structure was found at 52.8 Km (Fig. 5.4). Note that one distance class (33 Km), the analysis could not be performed due to lack of pairwise comparisons.

SEN males followed the same pattern as found for GB66 males and females of significant positive spatial structure at short distances (6.6 Km and 26.4 Km). At larger distances, SEN males display significant negative spatial structure (39.6 Km, 59.4 Km and 66 Km). For SEN females, only one distance class (at 6.6 Km) displayed significant positive autocorrelation and one distance class (at 26.4 Km) showed significant negative autocorrelation (26.4 Km). Note that for three distances classes for males (46.2 Km and 52.8 Km) and three classes for females, the analysis could not be performed due to lack of pairwise comparisons (Fig. 5.4).

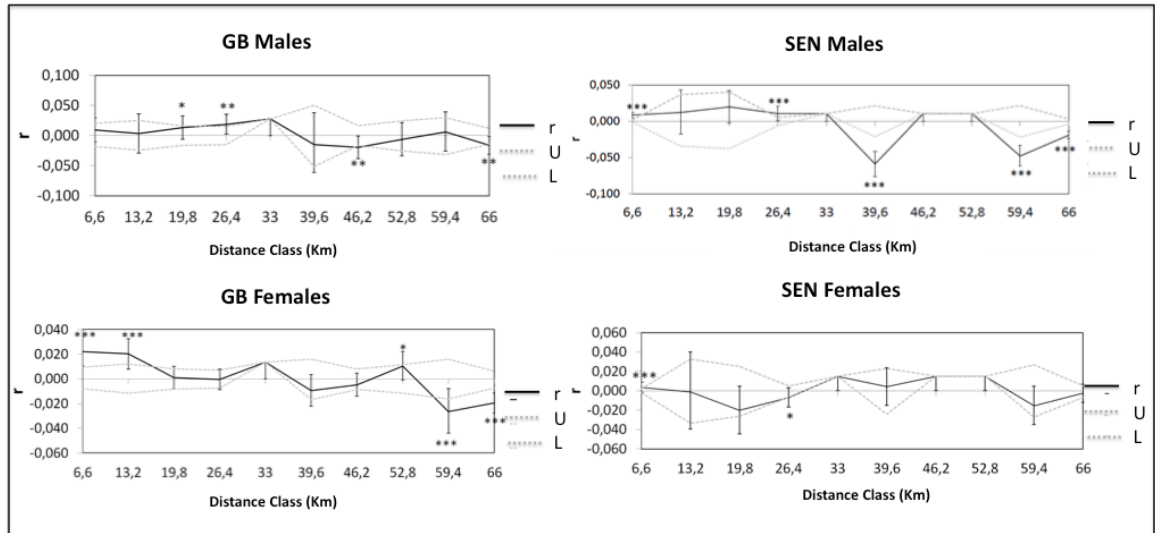


Figure 5.4: Results of the spatial autocorrelation analysis for GB (Guinea-Bissau) and SEN (Senegal) males and females. r is the correlation coefficient between genetic and geographic distance at ten distance classes (end point). U and L are upper and lower limits for the 95% confidence band under the null hypothesis of random distribution of genotypes across the landscape. Error bars represent 95% confidence intervals around each mean correlation coefficient. Distances classes with significant pairwise genetic distances are the ones standing outside the dashed line and highlighted. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Within GB66, the spatial autocorrelation was not significantly different between males and females (Total Omega for data = 2.17, $p = 0.83$) (Fig. 5.5). Both sexes present a pattern of higher positive spatial autocorrelation at short distances and achieve more negative spatial autocorrelation at larger distances (66 Km) (Fig. 5.5). In SEN we found an acute difference between sexes in the spatial autocorrelation pattern (Total Omega for data = 18.19; $p = 0.0001$). SEN males displayed significantly higher positive spatial autocorrelation at 34 Km ($p = 0.019$) and lower at 50Km ($p = 0.0001$) and at 66 Km ($p = 0.007$) than females (Fig. 5.5). Females displayed a lower level of spatial structure when compared with males, a result concordant with the STRUCTURE analysis.

The spatial autocorrelation comparison between GB66 and SEN males showed a similar pattern and was not significantly different (Total Omega for data = 3.7; $p = 0.49$) (Fig. 5.5). In both sampling locations, the greatest decrease in r is achieved between 33 Km and 50 Km, increasing slightly at 66 Km (Fig. 5.5). The largest difference between GB66 and SEN males lay in the 50 Km distance class, where the SEN males were significantly more dissimilar than the GB males ($p = 0.032$). This result was concordant with the comparison of the STRUCTURE analysis between GB66 and SEN males, in which Guinean-Bissau males were not genetically structured.

The pattern of spatial autocorrelation between SEN and GB66 females was quite different (Total Omega for data = 12.65; $p = 0.001$) (Fig. 5.5). The autocorrelation coefficient for GB66 females, r decreases almost in a linear fashion when compared with SEN females r . GB66 females seem to be significantly more similar at short distances ($p = 0.002$) and more dissimilar at larger distances ($p = 0.022$) (Fig. 5.5).

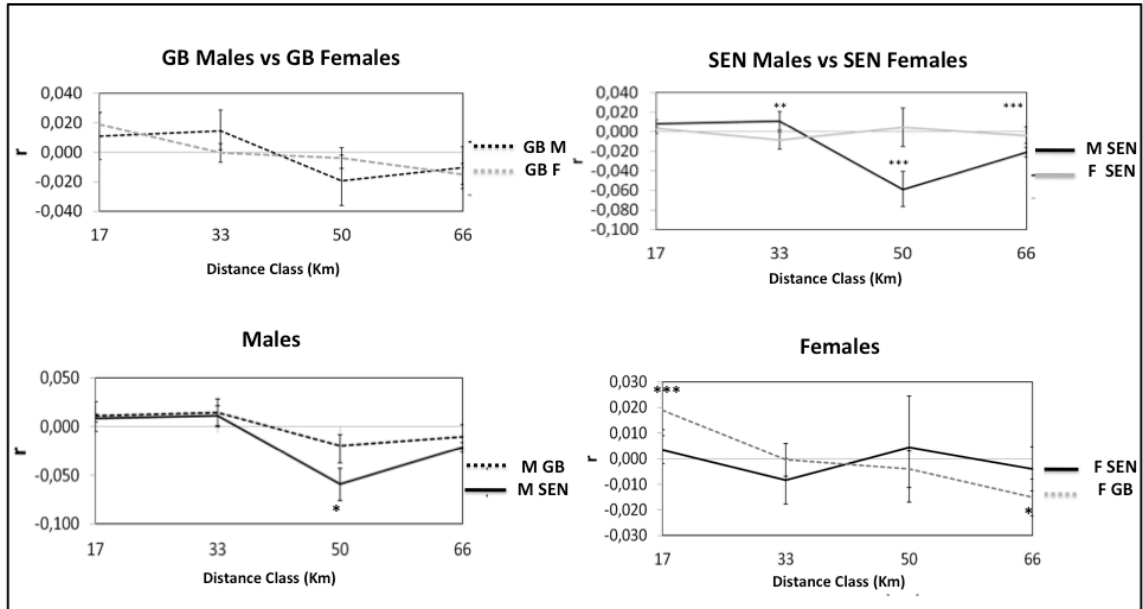


Figure 5.5: Results of the spatial autocorrelation comparison between GB (Guinea-Bissau) males and females (top left) and SEN (Senegal) males and females (top right), between GB and SEN males (bottom left) and between GB and SEN females (bottom right). r is the correlation coefficient between genetic and geographic distance at in ten distance classes (end point). Distances classes in which a significant pairwise genetic distances is found between the compared groupings are highlighted. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

5.4.4.3 – *Comparison between GB and SEN social units*

Reflecting the sampling of related individuals within social units, the mean relatedness within the social units was relatively high in most of the cases (Fig. 5.6). The highest values were obtained in the Cambeque (QGM_r = 0.5) and the Canamina (QGM_r = 0.4) social units. The SEN social units pairwise relatedness did not vary considerably across the social units considered (QGM_r = 0.1 in Simenti to QGM_r = 0.2 in all other social units). In contrast, we found a higher variation in relatedness within social units in GB66 (QGM_r = 0.5 to 0) (Fig. 5.6). The Bakar Contê social unit (sampled in Cufada) displayed lower and non-significant mean pairwise relatedness (QGM_r = 0.0; $p = 0.16$) evidencing sampling of non-related individuals within this social unit. In average, the mean pairwise relatedness within SEN social units (Rel = 0.07; Rel_c = 0.131) was not

significantly different from GB66 ($\text{Rel} = 0.05$; $\text{Relc} = 0.035$; two sided-test based in 100 permutations, $p_{\text{Rel}} = 0.67$ and $p_{\text{Relc}} = 0.14$).

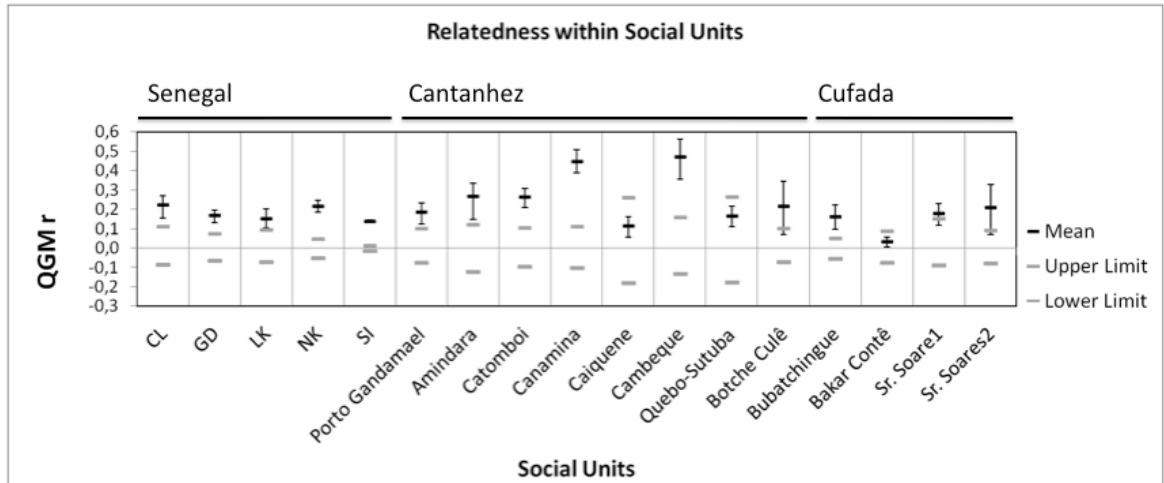


Figure 5.6: Within group mean pairwise relatedness estimated using Queller and Goodnight estimator (QGMr). Grey lines represent the permuted 95% confidence intervals (Upper and Lower limit) around the null hypothesis of zero relatedness. Significant mean pairwise relatedness is standing outside the Upper limit. Error bars represent bootstrapped confidence intervals around the mean coefficient of relatedness

Male mean pairwise relatedness within social units was overall high and significant, although two social units displayed non-significant values (Fig. 5.7). It varied between $\text{QGMr} = 0.4$ (Catomboi) to $\text{QGMr} = -0.04$ (Bakar Contê) and $\text{QGMr} = -0.027$ (Sr. Soares 1) (Fig. 5.7). In average, the males sampled in the GB66 social units were less related than the males in Senegalese social units ($\text{Relc}_{\text{GB66}} = -0.194$ and $\text{Relc}_{\text{SEN}} = 0.113$, two-sided test, based in 1,000 permutations, $p = 0.04$) (Fig. 5.8). Although the social units with lowest mean pairwise relatedness were mainly sampled in Cufada, in average, we did not found a significant difference between males sampled in Cantanhez and Cufada ($\text{Rel}_{\text{Cantanhez}} = 0.011$, $\text{Relc}_{\text{Cantanhez}} = -0.135$; $\text{Rel}_{\text{Cufada}} = -0.039$, $\text{Relc}_{\text{Cufada}} = -0.236$, two sided-test based in 1,000 permutations, $p_{\text{Rel}} = 0.55$ and $p_{\text{Relc}} = 0.142$) (Fig. 5.8).

The mean pairwise relatedness within social units estimated for females was again in most cases high and significant, with the Canamina social unit displaying the highest figure ($\text{QGMr} = 0.42$), followed by Cambeque ($\text{QGMr} = 0.34$) (Fig. 5.7). The Bakar Contê social unit showed once again the lowest and non-significant figure ($\text{QGMr} = 0.07$, $p = 0.15$). On average, females sampled in GB66 social units were as related as females sampled in the SEN social units ($\text{Relc}_{\text{GB66}} = 0.03$ and $\text{Relc}_{\text{SEN}} = 0.06$, two sided test, based in 1,000 permutations, $p = 0.5$) (Fig. 5.8). No differences were found in the relatedness between females sampled in Cufada and Cantanhez social units ($\text{Rel}_{\text{Cantanhez}} = 0.049$,

$Rel_{Cantanhez} = 0.084$; $Rel_{Cufada} = 0.046$, $Rel_{Cufada} = 0.185$, two sided test based in 1,000 permutations, $p_{Rel} = 0.93$ and $p_{Rel} = 0.31$).

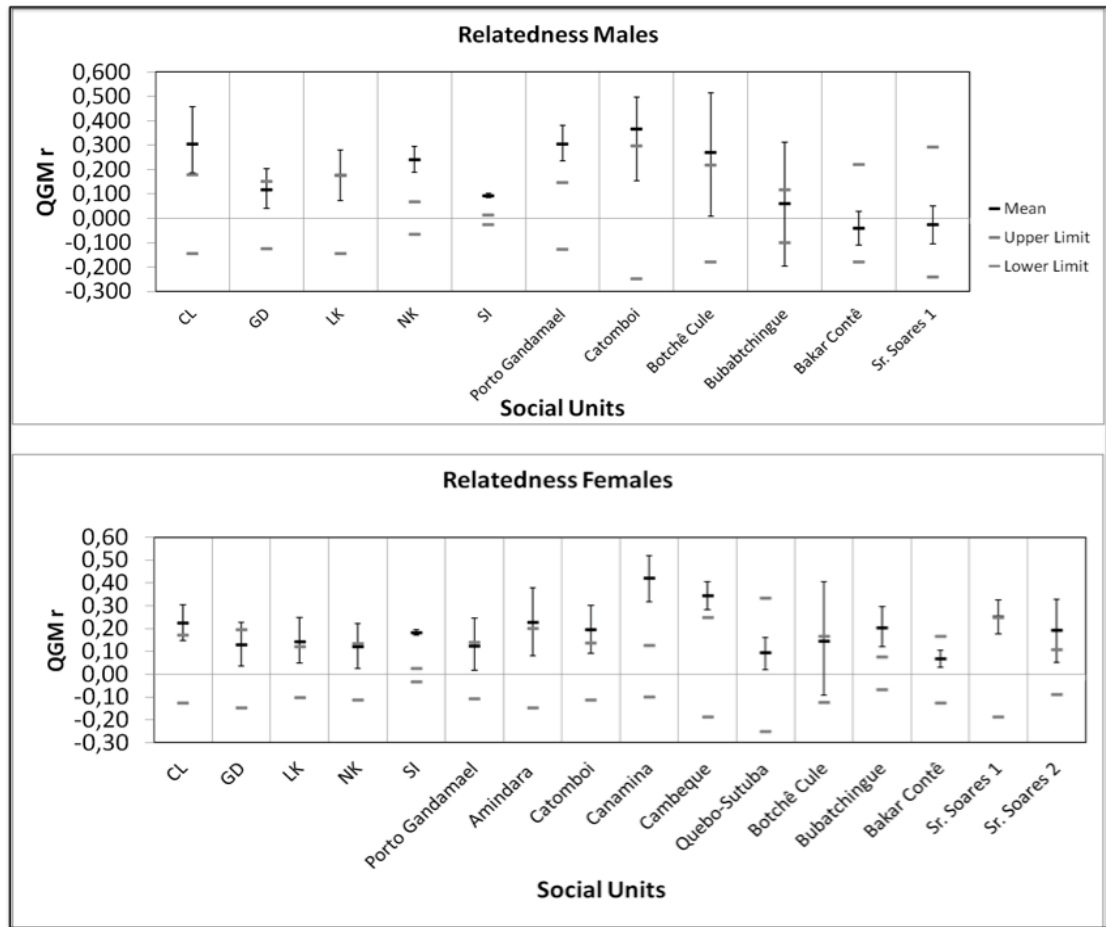


Figure 5.7: Male and Female's within group mean pairwise relatedness estimated by the Queller and Goodnight estimator (QGMr). Grey lines represent the permuted 95% confidence intervals (Upper and Lower limit) around the null hypothesis of zero relatedness. Significant mean pairwise relatedness is standing outside the Upper limit. Error bars represent bootstrapped confidence intervals around the mean coefficient of relatedness.

The differences in genetic diversity and inbreeding between SEN and GB66 social units are related with males rather than females. GB66 males displayed significantly lower observed heterozygosity ($Ho_{GB66 \text{ males}} = 0.52$) and higher and positive *Fis* values ($Fis_{GB66 \text{ males}} = 0.09$) than the SEN males ($Ho_{SEN \text{ males}} = 0.62$ and $Fis_{SEN \text{ males}} = -0.06$) (Fig. 5.8). *AR* and *Hs* did not vary significantly (Fig. 5.8). Nevertheless, *Fst* between male GB groups seems to be lower than in SEN ($Fst_{GB66 \text{ males}} = 0.01$ and $Fst_{SEN \text{ males}} = 0.05$). Females did not differ significantly between SEN and GB66 social units (Fig. 5.8).

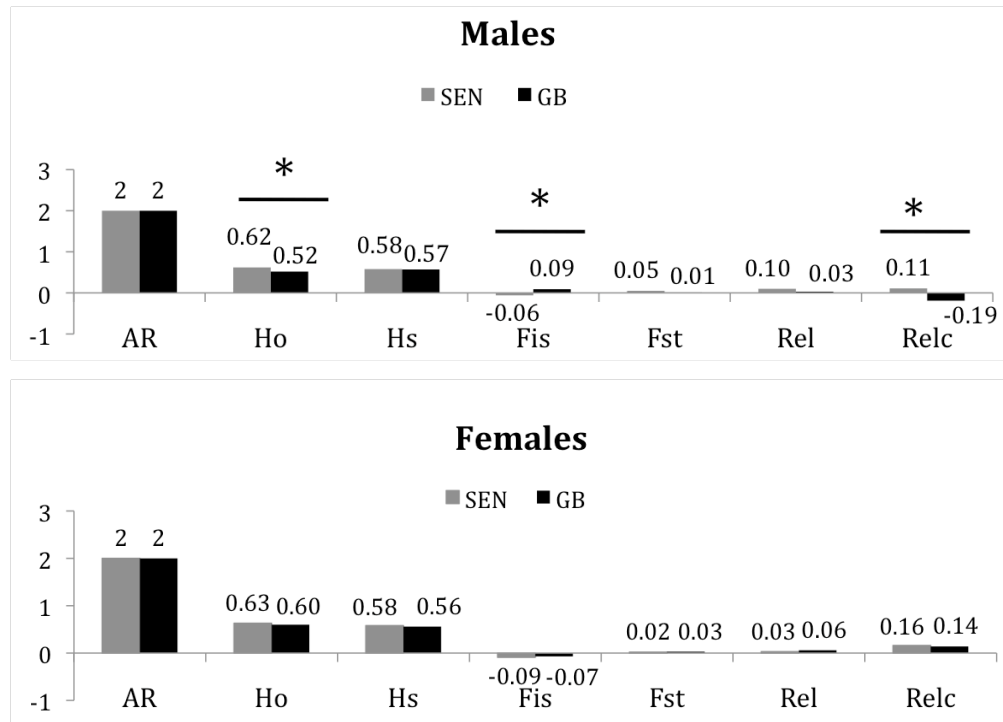


Figure 5.8: Comparison between mean within groups between SEN and GB males (top) and females (bottom). *AR* (allelic richness), *Ho* (observed heterozygosity), *Hs* (within group gene diversity), *Fis* (coefficient of inbreeding), *Rel* (relatedness) and *Relc* (relatedness unbiased). Significant differences were tested by a two-sided test using 1,000 permutations (assuming that males were the most philopatric sex) and are highlighted. * $p < 0.5$

5.4.4.4 – Comparison between males and females in GB and SEN

In the comparison between males and females within SEN and GB66 social units we also found significant differences. In GB66 social units, males were significantly more inbred than females and displayed significantly lower *Ho* (Fig. 5.9). No significant differences were found in levels of relatedness, *Hs* and *Fst*. In contrast, within SEN social units, males displayed significantly higher levels of relatedness and higher *Fst* values when compared with females. No differences were found in levels of inbreeding, *Ho* and *Hs* (Fig. 5.9).

The significant differences found within GB66 social units were related with Cufada and not Cantanhez. Cufada males displayed significantly higher and positive levels of *Fis* and higher levels of *Hs* when compared with females (Fig. 5.10). Although not significantly, males tended to be less related than females. This trend was also found in Cantanhez, although not significant (Fig. 5.10).

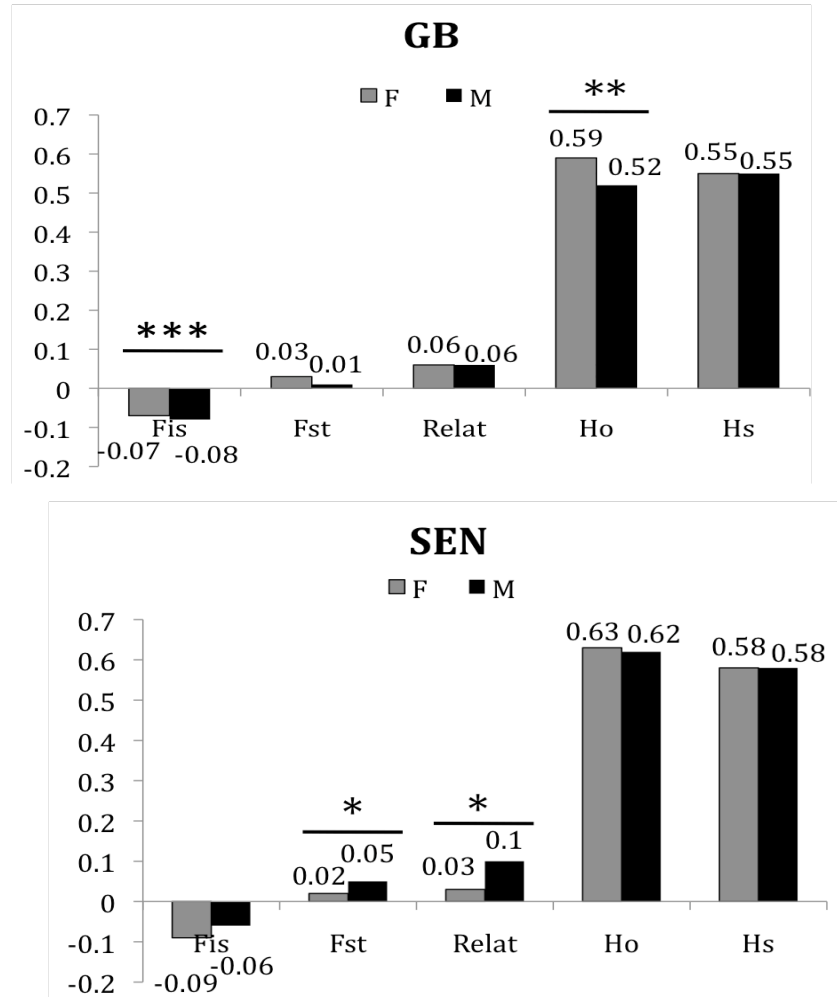


Figure 5.9: Comparison between males and females within social units sampled in GB (top) and Senegal (bottom). *AR* (allelic richness), *Ho* (observed heterozygosity), *Hs* (within group gene diversity), *Fis* (coefficient of inbreeding), *Rel* (relatedness) and *Relc* (unbiased relatedness). Significant differences were tested by a two-sided test using 1,000 permutations (assuming that males were the most philopatric sex) and are highlighted. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$

5.4.4.5 - *mAIC* and *vAIC*

The *mAIC*, as estimated by GenAlEx, was negative for GB66 and SEN males (*mAIC*_{GB66 males} = -0.37; *mAIC*_{SEN males} = -0.20) and positive for GB66 and SEN females (*mAIC*_{GB66 females} = 0.19; *mAIC*_{SEN females} = 0.28). The *vAIC* was slightly greater for GB66 males than SEN males (*vAIC*_{GB66 males} = 0.34; *mAIC*_{SEN males} = 0.20) and similar between GB66 and SEN females (*vAIC*_{GB66 females} = 0.20; *vAIC*_{SEN females} = 0.17). Nevertheless, this analysis did not reveal significant differences in the distribution of assignment indexes between the sexes, for both GB66 and SEN (two-tailed U test, $p > 0.05$).

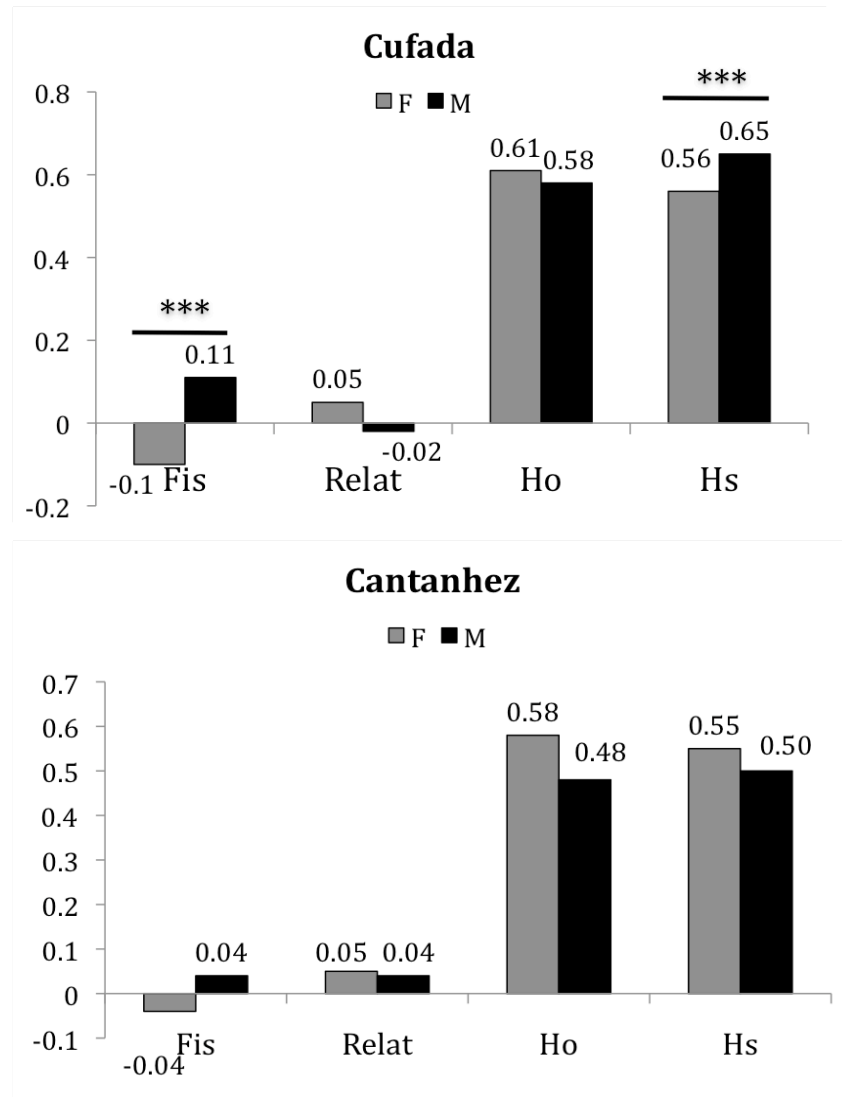


Figure 5.10: Comparison between males and females within social units sampled in Cufada (left) and Cantanhez (right). *AR* (allelic richness), *Ho* (observed heterozygosity), *Hs* (within group gene diversity), *Fis* (coefficient of inbreeding), *Rel* (relatedness) and *Relc* (relatedness unbiased). Significant differences were tested by a two-sided test using 1,000 permutations (assuming that males were the most philopatric sex) and are highlighted *** $p < 0.001$.

When the analysis was repeated for sampling locations within each country, a significant difference in the distribution of assignment indexes was found between the sexes in Cufada (GB66) (two-tailed U test, $p = 0.001$) (Fig. 5.11). Males in Cufada showed negative mean assignment index and greater $vAIC$ ($mAIC = -1.16$; $vAIC = 0.37$) when compared with females ($mAIC = 0.54$; $vAIC = 0.23$), which is what is expected if males were the dispersing sex and females were philopatric (Fig. 5.11). Eleven males out of 13 sampled in Cufada displayed negative AIC values. The sample CUBAK1234 (Fig. 5.11) presented the most negative value ($AIC = -4.17$). $mAIC$ between sexes for Cantanhez was non significant ($p = 0.25$), although females displayed a negative mean assignment index ($mAIC_{\text{females Cantanhez}} = -0.218$) when compared to males ($mAIC_{\text{males Cantanhez}} = 0.303$). On the

other hand, $vAIC$ did not vary considerably between the sexes in Cantanhez ($vAIC_{\text{Cantanhez females}} = 0.30$ and $vAIC_{\text{Cantanhez males}} = 0.40$).

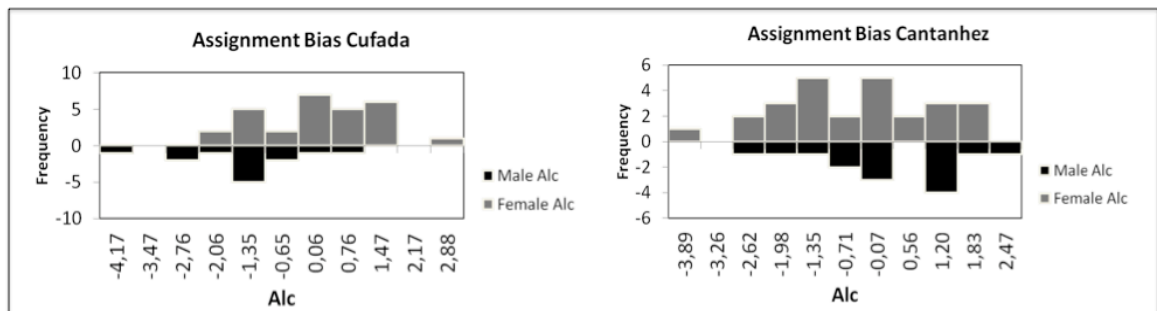


Figure 5.11: Distribution of the AIC for males and females in Cufada (left) and in Cantanhez (right). Note that in Cufada plot, the male CUBAK1234 show the most negative AIC.

5.4.5 - GB sex-biased dispersal patterns

BAPS spatial analysis including all samples in GB distinguished two clusters, consistently across runs (posterior probability of 0.99) in both males and females datasets. For males, Cluster 1 was formed by all samples collected in Cantanhez and in Cufada, with the exception of four Cufada samples that grouped in Cluster 2. Cluster 2 was also formed by all Boé samples. The Cufada samples that grouped in Cluster 2 were sampled in Bakar Contê (two samples), Guebambol (one sample) and sr. Soares 1 (one sample) (Fig. 5.12). For females, a similar pattern was observed. BAPS spatial analysis grouped in Cluster 1 all samples collected in Cufada and Cantanhez with the exception of three samples collected in Cufada (one in Bakar Contê, one in Bubatchingue and one in sr. Soares 1) that were grouped in Cluster 2. Cluster 2 also included all samples from the Boé region (Fig 5.12). The majority of males and females sampled in Cufada were grouped in Cluster 1 (76 % and 91.1 %, respectively). However, the proportion of males and females grouped in Cluster 2 in Cufada was quite different: 24 % of males contrasting with only 8 % of females.

The analysis performed in STRUCTURE produced concordant results with BAPS analysis within GB for males but not for females. In males, $K = 2$ was the clustering solution with the highest modal value in the ΔK distribution (Evanno *et al.*, 2005) and the clustering solution with largest Log (likelihood) and highest posterior probability (Posterior Probability_{K2} = 1) (Fig. 5.12). The individual q values were nearly continuous (not shown), therefore individuals were assigned to each cluster when $q > 0.80$ and all other individuals were treated as a product of admixture between clusters (Table 5.5).

Cufada clearly displayed a higher proportion of admixed individuals (82.4 %) when compared with the other sampling locations (54 to 46 %, Cantanhez and Boé respectively). Nevertheless, the proportion of individuals assigned to Cluster 1 and 2 is still considerable (6 % and 12 %, respectively) (Table 5.5). For females, no population genetic structure was detected by this analysis, even though the STRUCTURE graph pointed to a clear separation between the females sampled in Boé and all other sampling locations (Fig. 5.12). The clustering solution with the largest Log (likelihood) and highest posterior probability was when $K = 1$ (posterior probability $_{K1} = 0.99$), although the highest modal value in the ΔK distribution (Evanno *et al.*, 2005) was six, followed by two (Fig. 5.12).

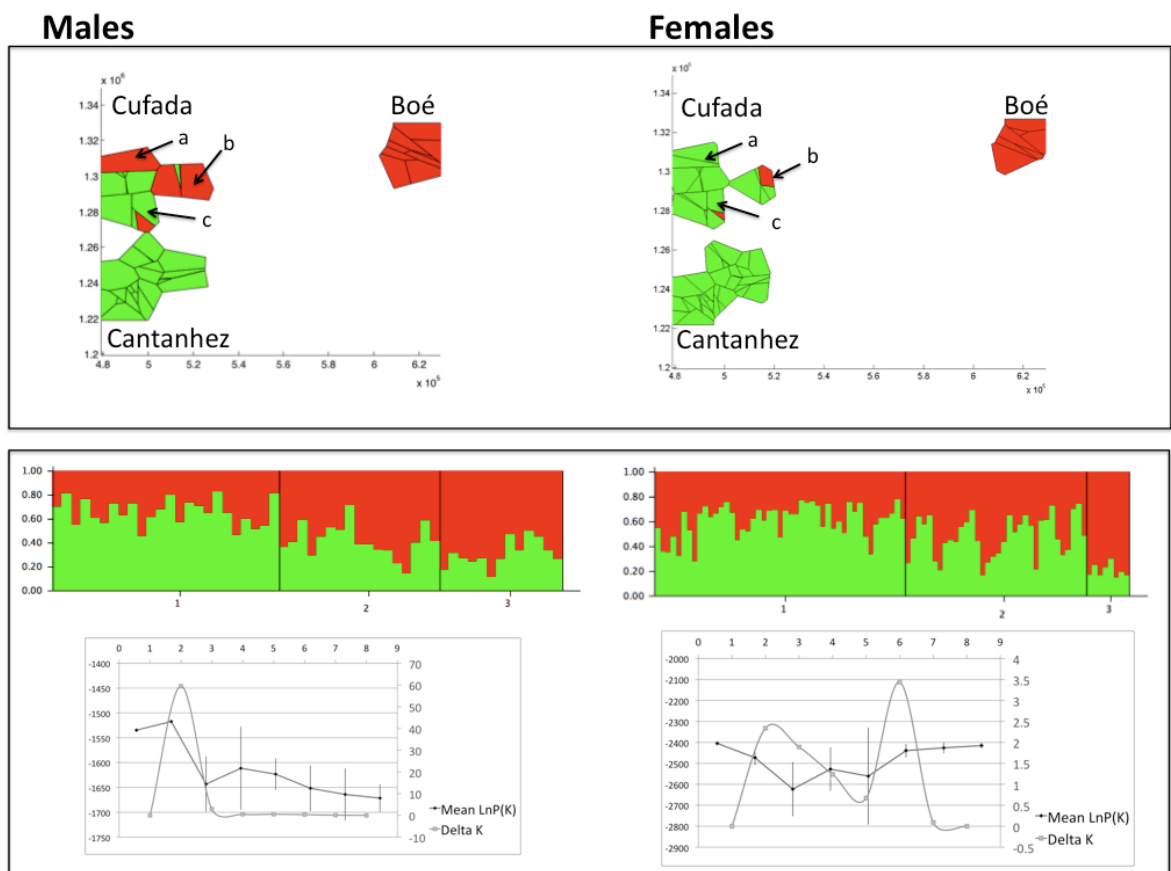


Figure 5.12: Above: results of the BAPS analysis for Guinea-Bissau females and males separately. The number of different colours represents the number of clusters found by this analysis. Letters indicate social units in Cufada where individuals assigned to Cluster 2 were found a) Guebambol, b) Bakar Contê, c) Sr. Soares 1. Below: Results of the STRUCTURE analysis for Guinea-Bissau males and females. In the graphs, a single vertical bar represents a baboon. The colours show the q membership of individuals in the two clusters. The sampling location is indicated below STRUCTURE graphs: 1– Cantanhez, 2 – Cufada, 3 – Boé. The graphs on the bottom show DELTA K and Mean Ln P(K) values as a function of number of putative populations (K).

Table 5.5: Proportion of number of males (%) assigned to each cluster identified by STRUCTURE analysis per sampling location. N – sample size. Individuals were assigned to Cluster 1 or Cluster 2 if $q > 0.8$ or otherwise considered admixed.

Sampling Regions	N	Cluster 1	Admixed	Cluster 2
Cantanhez	24	45.8	54.2	0.0
Cufada	17	5.9	82.4	11.8
Boé	13	0.0	46.2	53.8

5.5 - Discussion

5.5.1 - Differences in number of males and females

A significant difference was found in the proportion of males and females across the SEN and GB sampling locations. In GB social units, nearly twice as many females were sampled in the Cufada and Cantanhez social units. We excluded the possibility of high ADO that would fail to detect male individuals, as ADO for the sex determination protocol was only of 9%. A more likely explanation could be related to hunting practices. As male baboon carcasses have a higher economic value for hunters in GB, more males could be targeted. As a result, we could expect an effect on the demographic patterns within the sampled social units. This hypothesis correlates well with the higher number of males sampled at the bushmeat market (8 males out 14 samples), although such a small sample might not be representative of the quantities being traded.

The next most likely explanation could be an increased probability of sampling females. Social units described for the apparently multi-layered social organization of this subspecies (Sharman, 1981; Galat-Luong *et al.*, 2006; Patzelt *et al.*, 2011) were: the *One Male Units* (OMU) (e.g. one male adult and multiple adult females), the *Multi Males Units* (MMU) (e.g. multiple adult males and adult females) and the *Single Male Units* (SMU) (e.g. solely adult males). The most frequent unit observed in Senegal was the MMU, formed on average (\pm SD) by 23 (\pm 15.6) individuals: 3.8 (\pm 2.6) males and 9.1 (\pm 6.3) females [sex ratio 1:2.5 (\pm 1:1.1)] (Patzelt *et al.*, 2011). The low habitat visibility in GB prevented an accurate estimation of the group size or the social unit sampled. Considering the sampling success at each location (roughly 30 samples per site), the observation of more than one adult male in the groups and the similarity between the averaged sex ratio (Cantanhez: 1:2.3 and Cufada: 1:1.85) and Patzelt *et al.* (2011) results, it is possible that

the sampling in Cantanhez and Cufada consisted of MMUs. As a result, we would have proportionally sampled more females than males.

5.5.2 - Differences in social groups and sex-biased dispersal patterns

Evidence gathered by Fickenscher (2010) suggests a tendency for Guinea baboon dispersal patterns to be female biased. In SEN, when compared with males, females showed lower *Fst* values between subpopulations and lacked population genetic structure. In addition, females display lower average pairwise relatedness values and a weaker correlation between pairwise relatedness and geographical distance (Fickenscher, 2010). Complementary to Fickenscher's (2010) work, we found an acute difference in the spatial genetic structure between males and females in SEN. The results agree with Fickenscher's (2010) conclusion of less constrained female-mediated gene flow and a pattern of male philopatry.

In contrast in GB, such a clear sex-biased dispersal pattern within the same geographic scale was not found. We could not detect a clear difference in population structure between the sexes using both STRUCTURE and spatial autocorrelation analysis. In addition, we could not detect a significant difference in *Fst* and relatedness between males and females within GB, contrary to what was found in SEN (Goudet *et al.*, 2002).

Lack of detection of sex-biased dispersal can arise from a combination of factors related to sampling design (inclusion of pre-dispersers in the dataset, small sample size and geographic distance between sites), variability of genetic markers and differences between sexes in dispersal rates (Prugnolle and Meeus, 2002; Goudet *et al.*, 2002). In this study, the sampling strategy was comparable with respect to sample size ($N_{GB} = 111$ and $N_{SEN} = 165$), variability of genetic markers and distance in which bias was assessed. The inability to sample only adult individuals was analogous in both sites and is improbable a significant difference in the proportion of pre-dispersers individuals in the datasets. In addition, the inclusion of pre-dispersers individuals in the analysis did not hinder the exposure of a clear sex-bias dispersal pattern in SEN. The non-detection of such a clear pattern for GB most likely represents a true difference in the bias intensity of sex dispersal patterns.

The main difference between the populations is related with male dispersal. The male-mediated gene flow in SEN is somewhat more restricted when compared to the same

geographical scale within GB, where male gene flow seems to be stronger. The major difference is at 50 Km, a class corresponding broadly to distances between Cufada and Cantanhez. However, looking closely at the comparison between females in the spatial genetic structure, some differences do exist. In GB, females are genetically more similar at short distances and more different at largest distances, a typical pattern of philopatric individuals (Beck *et al.*, 2008). It could be argued that this difference is related with the different sampling schemes adopted (if for example, the smaller parties sampled in GB were formed by related females). However, we did not find a significant difference in the average female relatedness within social units between SEN and GB. Therefore, female-mediated gene flow in GB appears to be more limited, although not limited enough or only recently restricted to lead to significant population structure.

At a broader scale, by including samples from Boé, we identified a genetic structure for both sexes within GB. Overall, Boé was distinguished from the other sampling regions and individuals assigned to different clusters co-exist in Cufada. Cufada had been identified in a previous work (Chapter 4) as a contact area between genetically differentiated populations. A greater proportion of males, distributed across all social units in Cufada were identified as being genetically closely related to Boé. This result explains the different pattern found for Cufada males, when compared with Cantanhez, of significant positive *Fis* values (due to a Wahlund effect) and negative *mAic* (Goudet *et al.*, 2002). Boé males also displayed highly positive *Fis* values (Boé $Fis_{\text{males}} = 0.2$). On the other hand, Cantanhez area showed a tendency for female-sex bias dispersal as females displayed a negative mean assignment index when compared to males. Nevertheless, this difference was not significant.

As pre-dispersal individuals were included in our sample, it is not absolutely clear that all Cufada males with negative *Aic* and assigned to the different genetic clusters, emigrated during their lifetime. Some of the individuals sampled could be adult males or the offspring of emigrants. Also, the emigration of lactating females with dependent young to this area would lead to a positive signal of gene flow in the absence of dispersal by adult individuals (Schubert *et al.*, 2010). The great variation in the degree of admixture found in Cufada (82%) suggests that we sampled a mixture of first generation migrants and descendants of individual immigrants in Cufada. Considering the similar sample sizes ($N_{\text{males}} = 17$; $N_{\text{females}} = 23$) and equal probability in sampling a pre-dispersal individual of either sex, the results suggest higher rates of male immigration towards Cufada from a

genetically differentiated population. Nevertheless, this result should be taken with care as the sex ratio in baboon subspecies can be biased according with ecological conditions (Wasser and Norton, 1993).

5.5.3 - Possible explanations for differences between Guinea baboons populations in their sex-biased dispersal patterns

Several and complex causes can interplay to produce the different dispersal behaviour between GB and SEN males. The propensity for male philopatry showed in SEN may have been selected during the evolutionary history of this subspecies (Jolly, 2009). Jolly (2009) argued that the fast *Papio* genus range expansion (during the late Pliocene and Pleistocene periods) (Jolly, 2009) gave rise to unusually large troops. Males in these troops would benefit twofold from philopatric behaviour: while the large number of individuals hindered the probability of mating with related females, the unevenness and unpredictability of the habitat decreases male predisposition to leave the natal groups (Jolly, 2009). As a result of a higher reproductive fitness for philopatric males, the frequency of genes predisposing to philopatry would have increased (Jolly, 2009). Furthermore, habitat-related ecological variables and opportunities to breed could constrain the distance and rate of dispersal (Lawson Handley and Perrin, 2007). Density of conspecifics seems to be an important driving force in the emigration from natal groups (Bower and Benton, 2005), either acting as promoting (positive-density-dependent) or as a restrictive factor (negative-density-dependent) (Matthysen, 2005). Although in mammals, positive density dependent dispersal is quite common (Matthysen, 2005), in primate species and baboons populations in particular, negative density dispersal behaviours have also been reported (Smith, 1992; Alberts and Altmann, 1995; Strier, 2007).

Contrary to what was expected for GB males, SEN males live in big groups in an apparently high-density area (Byrne, 1981; Galat-Luong *et al.*, 2006). The competition between males of adjacent groups and the consequent higher probability of aggressive encounters, could limit the dispersal rate or distance for SEN males (Smith, 1992; Matthysen, 2005). Alternatively, the attraction for conspecifics (relatives or possible mates) could lower the rates of social emigration in highly populated areas (Matthysen, 2005). It is still not certain if Guinea baboons live in patriarchal societies (Fickenschner, 2010; Patzelt *et al.*, 2011) but if they do, male baboons should profit from the cooperation with male relatives against other groups of related males (Strier, 2007). Kin cooperation

for competition over females and protection against predators during foraging would increase the benefits of remaining philopatric (Strier, 2007; Matthysen, 2005; Lawson Handley & Perrin, 2007). In fact, males tend to remain philopatric in species with large groups sizes (Isbell, 2004), which seems to be the case for Guinea baboons (Dunbar, 1988).

The variation between SEN and GB dispersal patterns could be due to the great variability in the social dynamics suggested for this subspecies (Patzelt *et al.*, 2011; Galat-Luong *et al.*, 2006). In SEN, the composition of social groups, across time and space seemed to be inconsistent (Galat-Luong *et al.*, 2006; Patzelt *et al.*, 2011). This was justified as an adaptation to the habitat variation within the distribution area (Galat-Luong *et al.*, 2006), or to a new recently acknowledged type of social organization for the genus *Papio* (Patzelt *et al.*, 2011). As the Guinea baboon has been poorly studied (Henzi and Barrett, 2003; Galat-Luong *et al.*, 2006; Zinner *et al.*, 2009; Fickenscher, 2010; Patzelt *et al.*, 2011) at this point it would difficult to predict to what extent a pattern in which both sexes disperse is atypical for this subspecies. However, it is plausible to argue great adaptability from this subspecies under variable environmental conditions. If evolutionary mechanisms have selected for male philopatric behaviours in Guinea baboon (Jolly, 2009), GB females might be displaying the same propensity for dispersal as SEN females while GB males might be adjusting their behaviour to an anthropogenic-impacted environment.

The dispersal of males in GB could be explained in two ways. First, the conditions of higher predation risk in areas with intense hunting practices could increase the benefits for both males and females to disperse because the survival costs of remaining in the natal home range would be considerable (Waser *et al.*, 1994; Jack and Isbell, 2009). Therefore males may be escaping from hunters to less disturbed areas. For example, Alberts and Altman (2001) discussed the increased human pressure in Mount Kilimanjaro as one possible reason to induce immigration of Anubis into Yellow baboons at Amboseli.

The second explanation is related to hunting-driven altered sex ratios and age structure, which seems to greatly influence condition-dependent dispersal strategies in other hunted species (Loveridge *et al.*, 2007; Costello *et al.*, 2008; Pérez-González and Carranza, 2009; Pérez-Espona *et al.*, 2010). Male dispersal in primate species can be a means to increase their reproductive outcome (Isbell, 2004). Male movements would be stimulated by either a lower availability of females within their natal groups or in their

home areas (Rasmussen, 1979; Altmann, 1990; Smith, 1992; Isbell, 2004; Strier, 2007) or towards areas with greater accessibility to cycling females (Smith, 1992; Clarke *et al.*, 2008; Carnes *et al.*, 2011). Baboon male dispersal could be either in the shape of social or location dispersing behaviours (Altmann, 1990) or by the differential use of the home range area (Rasmussen, 1979).

If the GB population sex ratio is biased towards females, areas depleted of adult male baboons in GB could induce a “vacuum effect”. Immigrant males would occupy those vacant areas (Ji *et al.*, 2001; Perrin and Goudet, 2001), an effect observed in other hunted and but territorial species (Jedrzejewski *et al.*, 2005; Carter *et al.*, 2007; Loveridge *et al.*, 2007). High hunting-driven mortality of male baboons is suggested by the high economic return value of male carcasses in urban bushmeat markets (Chapter 3) and apparent preference of hunters towards those individuals (Chapter 3; Cá, 2008). Additionally, a significant female-biased sex ratio in GB social units suggests lower density of male baboons.

As a result of such a “vacuum effect”, when compared with females, GB males should feature the largest dispersal distances. This hypothesis correlates well with the finding of a higher proportion of males assigned to a genetically differentiated population in Cufada. Also, within Cufada social units, males were on average unrelated when compared with females, contrary to what was found in SEN groups. This may suggest that females dispersed from neighbouring groups (e.g. kin-based dispersal, Ji *et al.*, 2001) while males tend to disperse from areas located further away (Ji *et al.*, 2001). Settlement of dispersing males in Cufada implies a previous decline in density of male baboons in that area. We do not have any evidence on the intensity of hunting targeting males in Cufada, but interviews with hunters suggest a preference to target male baboons preferentially (Cá, 2008; Chapter 3). As this area was a hunting reserve before the establishment of the park (in 1999) (Ferreira, 1948) and is located next to the only road connecting the south of the country to the capital and used to transport the bushmeat trade (Casanova and Sousa, 2007), hunting practices were probably common in the past (Ferreira, 1948; Casanova and Sousa, 2007; Chapter 3).

However, the observation of juveniles kept as pets throughout GB (Hockings and Sousa, 2011) points to a hunting-driven adult female mortality. Lower number of females within groups could increase mating competition between males and induce movement

between groups. Secondary male dispersal in primate species has been observed when mating competition was high (Jack, 2003), particularly, if immigrant males have reduced reproductive success in the second social group where they arrive (Alberts and Altmann, 1995).

5.5.3.1 - The Cufada case and implications for conservation

Cufada groups seem to be formed by a high proportion of unrelated individuals. This pattern has already been found in social groups of other hunted social species (Nyakaana *et al.*, 2001; Jedrzejewski *et al.*, 2005; Gobush *et al.*, 2009). This finding implies important consequences for the conservation of this population. As individuals are forced to interact with non-kin, the quality of social bonds could be reduced (Gobush *et al.*, 2009; Archie and Chiyo, 2011). With the decline in affiliative interactions and with the increase in inter-individual competition (Gobush *et al.*, 2009), individuals are prone to higher stress levels (Gobush *et al.*, 2008). This increase in stress may lead to a lower number of offspring and therefore a negative outcome on reproductive fitness (Gobush *et al.*, 2008). In baboons, social unsteadiness, namely the take-over of the alpha position by immigrant males, has been shown to elevate stress-related hormones (Beehner *et al.*, 2005; Bergman *et al.*, 2005). Additionally, with the inclusion of new adult males in baboon social groups, infanticide events can become more frequent (Palombit, 2003). Immigrant males' takeovers frequently induce infanticide events across baboon groups (Collins *et al.*, 1984; Tarara, 1987; Swedell and Tesfaye, 2003). This could directly impact on population growth via the mortality of dependent young (Swenson *et al.*, 1997) or by increasing stress levels in lactating baboons females, which have been shown to be correlated with infanticide events (Engh *et al.*, 2006). Therefore, Cufada population should be monitored as these effects can severely stress the population.

This study emphasizes the importance of broad sampling strategies and the comparison between different populations when assessing the impact of human activities. The suggested acute alteration on a primate species dispersal patterns driven by hunting pressure is a cause for concern. Many primate species worldwide and in particular, the Guinea baboon subspecies (Starin, 1989; Galat *et al.*, 1999-2000; Oates *et al.*, 2008) are severely threatened by hunting practices. Along with population decline, this study suggests other impacts to Guinea baboon populations and stresses great conservation concern with this subspecies.

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Chapter 6 General Discussion

Sorti di pecadur sta na si sola di pe

(Luck is on the foot of mankind - Guinea-Bissau popular saying)

In this dissertation, I investigated genetic changes induced by hunting pressure at the population level in a generalist primate species. This study was focussed in the Guinea baboon subspecies in Guinea Bissau, a population mainly hunted for the meat trade. I estimated the genetic diversity and investigated hunting-related changes in population structure and in dispersal patterns. The hypotheses tested in this study were: 1) the population has undergone recent hunting-driven changes in its structure; 2) anthropogenic hunting affects dispersal behaviour; 3) relatedness and the sex ratio in social groups are affected by hunting practices. To address those hypotheses, I collected faecal samples in southern Guinea Bissau from social units at three different locations (Cantanhez Woodlands National Park, Cufada Lagoons Natural Park and the Boé region) and used two genetic markers (microsatellite loci and mtDNA). The sex of the individuals was determined using a molecular protocol.

6.1 – Overview of main results and further work

6.1.1 – Sampling and laboratory procedures

Non-invasive sampling in southern Guinea Bissau was carried out. Although it was not possible to find baboons in areas between sampling locations, groups were surprisingly easy to encounter in the Cantanhez Peninsula. They were frequently found at sites indicated by residents and guards (although using areas located at considerable distances from villages). The groups were quite silent, running away as I approached. At Cufada, the baboons did not escape.

The greatest difficulty of this project was the low amplification success for the microsatellite loci markers and low ratio of extracted samples with genotypes of sufficient quality to be included in the final dataset. I found empirical evidence for lower amplification success for samples collected in Cantanhez (77 %) when compared with Cufada and Boé (82 % and 80 %, respectively). Such difference was most probably related with the distinct dietary profiles of Cantanhez baboons, where samples were collected predominantly alongside the mangrove and possibly included a higher concentration of PCR inhibitors (Beja-Pereira *et al.*, 2009). Time constraints and the considerable costs associated with the genotyping process limited the number of repeats per locus/sample. The best approach found was to: 1) pre-select the samples with enough DNA quality or quantity to be

consistently genotyped for all loci; 2) to eliminate all the samples with the least reliable genotypes from the dataset; 3) to eliminate the least consistent locus (D21S1442). Despite the problems of non-amplification, I was able to include 149 samples in the final dataset, genotyped for fifteen loci, with considerably high quality in their genotypes (QI = 0.83). This approach allowed for a final ADO (11 to 32 %) and FA rates (1 to 5 %) to be in the range found in other primate studies (Fickenscher, 2010; Quéméré *et al.*, 2010) and assured quality of the data produced.

The pre-selection of faecal samples was only possible because the sampling efficiency was considerably high (557 samples in four months). Nevertheless, this approach could not be applicable in cases of rare and more elusive species, where only a small number of samples are usually collected. The high sampling success was achieved by involving the Park's residents, guards and guides in this project. The aims and possible implications of this work were diffused to the community using local radios, by contacting with the village's leaders and by co-organizing workshops, where scientific investigation (and its benefits for the community) were explained. These actions were quite prolific and are recommended in field sites with similar characteristics.

6.1.2 – Hunting pressure towards Guinea-Bissau primates

Previously, it was suggested that the species most frequently targeted for the bushmeat trade in Guinea Bissau were *P. h. papio*, *C. sabaeus* and *Colobus* monkeys (*Procolobus badius* and *Colobus polykomos*) (Casanova and Sousa, 2007; Cá, 2008). Residents were reluctant to provide informative statements and the urban markets were inaccessible to foreigners because primate hunting is illegal in the territory. Despite these conditions, the GB urban bushmeat trade was followed for the first time. We found 150 carcasses from six primate species being traded. The suspicion of widespread hunting practices towards primates (Gippoliti and Dell'Omo, 2003; Casanova and Sousa, 2007) was confirmed, however the species-specific contribution to the trade was unexpected. We found that *C. sabaeus* and *C. campbelli* were the most frequently traded species (32.2 % and 30.6 %, respectively), followed by the Guinea baboon (19.4 %). *Colobus* monkeys, on the other hand, were not as frequently traded as initially thought (11.5 % - *P. badius* and 1.4 % - *C. polykomos*).

The use of a DNA barcoding approach was very effective in distinguishing between species with similar body mass or with interchangeable common names. Without the use of

molecular tools, *C. campbelli* would be considered the single most traded species and most importantly, the trade of *C. sabaeus* would be ignored. The molecular identification of species traded at bushmeat markets is not common (but see Olayemi *et al.*, 2011) and so far, these methods have been applied in a partial number of species (Palumbi and Baker, 1994; Roman and Bowen, 2000; Eaton *et al.*, 2010).

We estimated a minimum of 2,008 primate specimens being traded every dry season in GB. Further work should include an evaluation of the trade across years and ought confirm the suggested break during the rainy season. The GB trade seems to constitute a severe threat to *C. campbelli* and *C. sabaeus*, species that were over-looked in the past because they were considered abundant and widespread (Gippoliti and Dell’Omo, 2003). The hunting practices towards *C. campbelli* could also affect colobus monkeys (considered endangered species, IUCN 2010) as they are frequently found in interspecific association in GB (Gippoliti and Dell’Omo 2003). The high rate of the bushmeat trade predicts a rapid population decline or a source-sink compensatory system, as observed in Guinea baboons (see below). Therefore, a census should be carried out to identify areas where those species are still abundant or have suffered recent disappearances.

We also recommend a complete characterization of the primate bushmeat trade in GB. Hunted areas and trade routes in Guinea Bissau should be identified in order to prioritize conservation measures towards the most heavily hunted populations. In GB, the bushmeat trade is associated with a lack of job opportunities for young people and a low availability of domestic meat. Measures should be employed to: i) train locals to increase domestic meat production and preservation; ii) provide local hunters with alternative incomes to the bushmeat trade.

We found that the carcasses size was the main determinant of its price at the stalls. With respect to male baboons, their body mass influenced the price but not the trade rate. Higher rates of relatively smaller species (*C. campbelli* and *C. sabaeus*) can be explained either by their abundance when compared with baboons or by consumer preferences for their meat. Further investigation on consumer species-specific preferences at the urban centres should be carried out, as it could influence the species trade rates.

According with our results, chimpanzee’s pet trade is considerably profitable within GB. However, it is still very common to see Guinea baboons, Patas monkeys and Vervet monkeys being kept as pets within the country. The responsible authorities should address

the pet trade phenomenon. Animals live in poor conditions and this trade implies the mortality of several individuals within the groups. As recommended by Casanova and Sousa (2007), these animals should be moved to sanctuaries.

The hunting of baboons seems to be unsustainable nowadays but was probably higher in the recent past. This may have led to a population decline (Gippoliti and Dell'Omo, 2003; Casanova and Sousa, 2007) and/or to behavioural adaptations to hunting pressure (silent groups and avoidance of human settlements) displayed by the southern GB population. A complete description of primate behavioural adaptations to anthropogenic hunting practices could be carried out. Additional further work should include a population viability analysis (PVA), to evaluate the probability of extinction of GB baboons having in consideration the impact of hunting pressure.

6.1.3 – Effects of hunting pressure towards the Guinea baboon in Guinea-Bissau

I investigated Guinea baboon population structure and genetic diversity in southern GB, using two molecular markers (mtDNA and microsatellite loci). I expected to find genetic discontinuities concomitant with areas of human settlements (villages or the main road connecting the south of the country to the capital). As a result of demographic isolation and reduced effective population size, I expected the genetic diversity for this population to be reduced.

By using Bayesian clustering and fifteen microsatellite loci, I found that the most dominant pattern of genetic structure was two moderately differentiated genetic units ($F_{st} = 0.09$). The genetic diversity of the population (mean number of alleles = 4.24; $U_{He} = 0.58$) was not significantly reduced when compared with a non-hunted population in Senegal ($He = 0.59$; Fickenscher, 2010). Boé was the most differentiated area at both nuclear and mtDNA level. Nevertheless, the sampling locations do not seem to be isolated. On the contrary, the results suggested i) a historical female-biased dispersal pattern (e.g. the distribution of mtDNA haplotypes not related to the sampling locations, no significant isolation by distance, greater percentage of total variation present within social units and haplotypes shared by individuals separated from each other by more than 50 Km) and ii) more recent contact (e.g. high proportion of admixed individuals in all sampling locations as estimated using microsatellite data, genetic similarity between samples distanced at 115.5 Km apart and first generation migrants). Lack of isolation between sampling locations is

probably related to the capacity for this subspecies to cover large distances (40Km per day, Galat-Luong personal communication).

As observed in other hunted species (Harris *et al.*, 2002; Allendorf *et al.*, 2008; Allendorf and Hard, 2009), past and current hunting-driven mortality of baboons seems to have induced genetic changes in hunted sub-populations (see Table 6.1). I found a contact zone (where gene-flow seems to be unidirectional and where admixed individuals are in higher proportion) and concordance in the location of genetic discontinuities for both genetic markers. These results suggest recent admixture of allopatrically differentiated populations. Higher immigration rates towards Cufada, probably not related with differences in habitat, could be the result of decreased local abundance (Ji *et al.*, 2001; Perrin and Goudet, 2001). Alternatively, the individuals may have been escaping to the protected areas.

The pattern of genetically differentiated baboons co-existing in the same social unit is similar to that found in hunted populations of other species (Jedrzejewski *et al.*, 2005; Gobush *et al.*, 2009). Hunting practices seems to have induced a demographic ‘sink’. To the best of our knowledge, these effects have not been described so far for a primate population. Primates with reduced capacity to disperse over larger distances or more sensitive to anthropogenic alterations on the habitat might become isolated (e.g. Liu *et al.*, 2008).

I found genetic similarity between samples separated by 115.5 Km (microsatellite data), related with the presence of first generation migrants identified in Cufada. Cufada population seems to be recovering from a hunting-driven demographic decline through immigration from other locations. According with my results, these migrants were originated from Boé or from a genetically related population and arrived in Cufada by dispersing over long distances or by secondary dispersal, as observed on other subspecies [Yellow baboons (Alberts and Altmann, 1995), Hamadryas baboons; (Hammond *et al.*, 2006)]. Nevertheless, migration over smaller distances, by crossing the Corubal River might have occurred.

Due to time constraints during this project, sampling in the northeast area was not possible. The identification of the immigrants’ source is important for the conservation of Cufada baboons (Howe and Davis, 1991) and further work should identify the immigrants origin and dispersal routes. Therefore, the presence and abundance of baboon groups in the north-east of the country should be investigated. A previous census (Gippoliti and

Dell’Omo, 2003) pointed to recent disappearances in the northwest areas and close to Bissau, however it is unknown if baboons currently persist in Bafatá and Gabu (located in central and north-western GB).

Table 6.1: Resume of main findings

Hypotheses	Predictions	Results	Effect of hunting practices in Guinea Baboons
1) The population has undergone recent hunting-driven changes in its structure	a) Demographically isolated sub-populations in heavily hunted areas; b) Reduced genetic diversity;	1) The genetic diversity of the southern population was not significantly reduced; 2) Recent contact between sampling locations; 3) Differentiated individuals co-existing in the same social unit;	1) Induction of contact zone (gene-flow unidirectional and where admixed individuals are in higher proportion); 2) Recent admixture of allopatrically differentiated populations; 3) Demographic sink;
2) Anthropogenic hunting affects dispersal behaviour	c) Different sex-biased dispersal pattern than non-hunted populations	4) Gene flow mediated by both sexes in hunted population; 5) Influx of males towards Cufada;	3) Males are dispersing in GB but not in Senegal. 4) Possible “vacuum effect”
3) Relatedness and the sex ratio in social groups are affected by hunting practices	e) Different patterns of sex ratio; d) Disrupted social groups;	6) Sex ratio was female-biased in the Cufada and Cantanhez social units; 7) Social units formed by unrelated individuals;	5) Possibly higher mortality levels for males than females; 6) Possibly disruption of social structure;

I compared Guinea-Bissau (GB) with Senegalese baboons (SEN) from the Parc National du Niokolo Koba, a population that has increased in size and currently suffers from little hunting pressure. I used a molecular sex determination protocol and thirteen common microsatellite loci (and similar laboratory procedures), to investigate differences between social units and compared sex-specific patterns of gene flow.

I found a significant difference between SEN and GB social units in the proportion of males and females. Nearly twice as many females were sampled in the Cufada and Cantanhez social units. This result can be explained by hunting-driven demographic

changes in the social units. Yet, differences in sampling strategy between GB and SEN could also potentially account for this pattern. Conditions of weak visibility in the field hinder counting of animals in the groups and identification of GB group's social dynamics. Nevertheless, the distinction between these two scenarios would be very important to correctly evaluate the demographic effects of hunting practices in GB.

Results suggested an impact on the dispersal behaviour of baboons caused by hunting. This study highlights the importance of broad geographical sampling and comparison between close related populations, when investigating dispersal patterns. Studies focussing on only one population might lead researchers to confound normative patterns with behavioural adaptations to anthropogenic-driven environmental changes.

I found a less intense sex biased dispersal pattern in GB. Male-mediated gene-flow seems to be more restricted in SEN but stronger in GB. Three environmental drivers could potentially induce the pattern observed: i) emigration by both males and females to escape hunters, ii) “vacuum effect” caused by hunting-driven decrease in male baboon density/number and iii) lower availability of females in social groups. The arguments supporting these three hypotheses are discussed below.

Intense hunting practices could induce emigration by both sexes because the survival costs of remaining natal would be higher (Waser *et al.*, 1994; Jack and Isbell, 2009). In this case, both GB males and females may be escaping from hunters to areas considered less disturbed (as observed by Alberts and Altmann, 2001), such as protected areas. In fact, the areas located between sampling regions have a lower density of baboons (no samples were found here, Chapter 4) and were referred as potential hunting areas (Chapter 3). Additionally, the location of genetic discontinuities (Chapter 4) suggests recent migration to sampling areas. Nevertheless, baboons inhabiting hunted areas between sampling regions might be displaying behavioural adaptations to the human's presence (as becoming more secrete), which would hinder their detection. As a result, this hypothesis would not be applicable.

Hunting-driven mortality of adult males and consequent bias of the population sex ratio towards females could induce immigrant males to move to vacant areas (a “vacuum effect”, Ji *et al.*, 2001; Perrin and Goudet, 2001), an effect observed in other hunted and territorial species (Jedrzejewski *et al.*, 2005; Carter *et al.*, 2007; Loveridge *et al.*, 2007). This hypothesis is the most plausible interpretation of the results of this project. Higher

hunting pressure towards males can be supported by the following arguments: i) higher price of adult baboon males at urban markets (Chapter 3); ii) statements by hunters referring to preference for adult baboon males (Chapter 3) and iii) GB social groups sex ratio significantly biased towards females when compared with a non-hunted population (Chapter 5). Hunting-driven depletion of baboon males would explain evidence for a demographic sink (Chapter 4) and male immigration towards Cufada (Chapter 5). The consequences of a male influx towards Cufada are social disruption, frequent infanticide events (Palombit, 2003) and increase of stress-related hormones levels (Beehner *et al.*, 2005; Bergman *et al.*, 2005). As a result, the population can decrease via mortality of dependent young (Swenson *et al.*, 1997) or may suffer a negative outcome in terms of reproductive fitness (Gobush *et al.*, 2008).

Hunting-driven mortality towards females could limit availability of females within natal groups or home areas and increase male-male competition (Rasmussen, 1979; Altmann, 1990; Smith, 1992; Isbell, 2004; Strier, 2007) and cause male baboons to emigrate from natal social groups. This hypothesis is only supported by the general observation of baboons being kept as pets throughout southern GB, which points to high mortality of adult females (Chapter 1). However, we did not find significant differences in the number of females in GB when compared with Senegal.

Even though a strong population structure for GB females was not identified, results suggest a weak or recent restriction on female dispersal between Cufada and Cantanhez. As a consequence of loss of habitat and continuous hunting practices, the two populations can become isolated. Therefore, further work should involve the estimation of migration rates between Cantanhez and Cufada using an ABC method (Csilléry *et al.*, 2010).

6.2 – Implications for GB primate conservation

Lack of an updated population assessment and basic knowledge on this baboon biology has hindered a correct evaluation of Guinea baboon conservation status (Oates *et al.*, 2008). The significant range contraction in other locations over the last 30 years (Oates *et al.*, 2008) emphasis the importance of the Guinea Bissau population for the global conservation of this subspecies. However, Guinea Bissau baboons display signs of a population disrupted by hunting practices.

Cufada baboon population seems to be recovering from a hunting-driven demographic decline through immigration from other locations. This population requires further monitoring as the contact between genetic differentiated individuals could bring negative consequences to its growth. Its maintenance also requires the recovering of ecological corridors connecting Boé to the southern areas. These corridors were already delimited by the national protected areas management institution (IBAP – Instituto para a Biodiversidade e Áreas Protegidas) but annually, locals burn the vegetation to cultivate dry land rice, sometimes constructing villages in those areas (Casanova and Sousa, 2007). This will hinder the movements of many species and increase mortality of primate groups during crop-raiding conflicts.

Cantanhez baboons are a conservation priority. A high density of villages delimits Cantanhez Park in the north and the habitat within the Peninsula is being lost at a rapid pace (Temudo, 2009). Baboons have recently disappeared or avoid areas in the north of the Peninsula (possibly due to the high concentration of villages) and are rare in adjacent areas (e.g. Cacine Peninsula). The evidence for recent contact between the Cantanhez and Boé population is weak, suggesting recent isolation from the northern area of the country. Cufada baboons might have been more affected by past hunting practices but in Cantanhez, baboons are currently forced to live in closely proximity to the numerous villages in the park. The apparent behavioural differences between Cufada and Cantanhez baboons suggest frequent contact with locals. Along with increased hunting pressure (for meat consumption, the bushmeat trade or as a result of crop-raiding conflicts), contact with humans could lead to a decline in the health of this population (Rui Sá, unpublished data). The most likely consequence is the extinction of baboons in the Peninsula. It is urgent to implement conservation measures at the north of the Cantanhez Peninsula. Appropriate ecological corridors between Boé region and Cantanhez should be design and a campaign of sensibilization focussing on the impacts of consumption and trade of bushmeat should be carried out in the northern located villages. Additionally, a restrict control of bushmeat trade in the Cacine Peninsula (and on the road connecting to Cantanhez Park entrance) should be employed. It has been mentioned by the interviewees that hunters from Cacine cross the river to hunt in Cantanhez. Therefore, hunting within the park should be prevented at all means.

Lack of coordination between institutions, parks residents, parks guards and guides, NGOs acting in the field and law enforcement agents has hindered the implementation of efficient conservation actions within southern GB parks.

Today, the Cantanhez park guards do not receive a salary for their functions, which promotes lack of authority in preventing illegal activities and corruption. The NGO AD (Acção para o Desenvolvimento) acting in Cantanhez area has been establishing a conservation programme for the last twenty years. Within the Park, fully protected areas, buffer zones and areas dedicated to farming were delimited. Additionally, an ecotourism program was established⁸. However, locals have difficulties in respecting the imposed rules (Temudo, 2009) and do not foresee the benefits of ecotourism (Costa, 2010). In Cantanhez, uncontrolled fires and timber extraction practices annually destroy a significant area of the original vegetation and increase buffer zones areas (Padrão, 2009) (see Fig. 3, Appendix 6). In Cufada the situation is not different. A small number of guards (eight) are responsible for law enforcement within the park area. The future of this area is uncertain, as the construction of a harbour for bauxite exportation destroyed one third of the vegetation and the Park might suffer disqualification of its protected status. For residents in both parks, the deficiency in i) compensation schemes, i) food safety, iii) annual variation of crops outcome and in iv) alternatives of income, probably encourage illegal hunting practices. The law enforcement is limited to the guards' actions and the most frequent situation is for the hunters to reoccur in their activities after being caught (Starin, 2010). Conservation actions should be articulated with urgency between all the stakeholders. The most likely outcome of the present situation is the defaunation of these areas.

This study sounds an alarm about the large scale at which hunting pressures can impact a primate population. Although baboons are usually considered an adaptable species, the findings of this study recommend a reassessment when designing and allocating conservation efforts. The gravity of the localized anthropogenic threats and in which manners the populations are compromised determines the species persistence. The actions reducing hunting practices in GB are urgent. Passiveness from the responsible authorities will result in the severe decline of the country's greatest patrimony: its biodiversity!

⁸ <http://www.adbissau.org/programas-nas-regioes/Cantanhez>

6.4 – References

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Chapter 7 Appendix 1 - The trade and ethnobiological use of Chimpanzee body parts in Guinea-Bissau: implications for conservation

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The Trade and Ethnobiological Use of Chimpanzee Body Parts in Guinea-Bissau: Implications for Conservation

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INTRODUCTION

Guinea-Bissau represents the western-most limit of the endangered West African Chimpanzee *Pan troglodytes verus* (Sousa *et al.*, 2005). During the 1980s, Chimpanzees were erroneously considered to be extinct in the country due to a total absence of information owing largely to political and civil unrest (Lee *et al.*, 1988). In 1994, a preliminary survey was conducted and the presence of Chimpanzees was reconfirmed (Gippoliti and Dell' Omo, 1995; 1996). More recently, research has been carried out in co-operation with national and local authorities, establishing a system for the systematic monitoring and management of this great ape (Casanova and Sousa, 2007). Within the country, Chimpanzees are distributed across the south of the Corubal River. Their presence is confirmed in two protected areas—Cantanhez National Park (CNP) and Cufada Lagoons Natural Park (CLNP) in the southwestern region—and in the eastern region of Boé (Casanova and Sousa 2007; Brugièrre *et al.*, 2009).

Due to high levels of exploitation, loss of habitat and habitat quality as a result of human activities, this subspecies is estimated to have experienced a significant population reduction in the past 20 to 30 years (IUCN, 2011). However, no recent data are available to allow for an estimation of rates of decline (IUCN, 2011). The most recent figures available, from 1996 (Gippoliti *et al.*, 2003), estimate that the number of Chimpanzees in Guinea-Bissau ranges from between 600 and 1000 individuals. It is estimated that Chimpanzee density in the southern area of CNP is of 2.34 nest builders/km² in a total area of 17.225 km², corresponding to 40 individuals (Sousa *et al.*, 2011), while in the neighbouring area east of Gadamael, just outside the CNP area, this value decreases to 0.89 nest builders/km² in a total area of

36.513 km², which corresponds to 33 individuals (Sousa, 2009). However, the exact number of individuals and communities for the whole CNP and the rest of the country remains unclear; with the aid of a molecular census, however, it will be possible to infer its effective population size (Sá *et al.*, 2009).

Anthropogenic disturbances such as habitat loss and fragmentation (e.g. logging activities and shifting land occupation for the purposes of agriculture and food production, e.g. cashew nuts), the hunting of infant animals for the pet trade, and casual deaths from crop raiding allied to extrinsic factors such as disease, are the main threats, not only to Chimpanzees but to all non-human primates in Guinea-Bissau (Gippoliti *et al.*, 2003; Casanova and Sousa, 2007; Brugièrre *et al.*, 2009). The species is classified by IUCN as Endangered, and listed in CITES Appendix I, and is also protected in Guinea-Bissau. Even though most primate species in Guinea-Bissau are traded for meat consumption, there is no evidence that this is the case for Chimpanzees (Minhos *et al.*, in prep.).

This paper reports on the use and trade of Chimpanzee body parts in Guinea-Bissau for traditional practices (e.g. for nutritional, medicinal or ritual purposes, or “animistic myths”). Informal interviews were conducted and observations made with a view to providing insight into how these human traditions and myths might pose an additional threat.

METHODS

Seven visits, of approximately four hours each, were made to Bandim market, the largest market in Bissau, the capital, during two weeks in September 2008 and a similar period in June 2010. Some 10–15 men were found to be offering wild animal body parts for sale (e.g. skin, bones, teeth, horns and scales). Where possible, morphological identification of the specimens viewed was made and photographs taken.

An ethnoprimate approach (i.e. the study of human and non-human primate interactions) aims to understand the incorporation of non-human primates into folklore, myths, the hunting of non-human primates for food, keeping non-human primates as pets, indigenous knowledge of non-human primate behaviour, among others (Wolfe and Fuentes, 2007; Fuentes and Hockings, 2010). In this study, the authors were interested in understanding and placing into context the social inclusion of Chimpanzee body parts for human traditional practices using informal interviews and ethnographic observations, although not enough data were collected to provide an in-depth analysis for such an approach.

Most of the vendors encountered were male. Five urban vendors in Bandim market and 17 rural informants in villages in the CNP and the Boé region were informally interviewed following an unstructured script, in order to document the geographical origin and use of Chimpanzee body parts, prices and the scale of the trade, i.e. whether at a national, regional, or transnational level. Direct observations of the trade were conducted in the market

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1. Leopard *Panthera pardus* (1a complete).
CITES Appendix I/IUCN Near Threatened;
2. Chimpanzee *Pan troglodytes verus*.
CITES Appendix I/IUCN Endangered;
3. Nile Crocodile *Crocodylus niloticus*.
CITES Appendix I/II/IUCN Lower Risk/Least Concern.
4. Guinea Baboon *Papio papio*.
CITES Appendix II/IUCN Near Threatened.
5. Possibly Lion *Panthera leo*.
CITES I/IUCN Vulnerable.
6. Possibly African Civet *Civettictis civetta*.
CITES Appendix III/IUCN Least Concern.
7. Antelope horns (species not identified).

Fig. 1. All animal-derived products for human traditional purposes in Bandim market, Bissau, Guinea-Bissau.

and field notes were taken. Informants were assured that the purpose of the work was not to condemn or report their practices to the local authorities. Every observation heard and/or seen was recorded and notes/interviews organized into social demographic categories (e.g. urban traders, local villagers, gender, ethnic group). Only information relevant to the research topic was assigned to these categories (Rubin and Rubin, 1995).

Only pieces of animal skins were seen for sale during the surveys (which could have derived from one or more specimens). As the vendors were reluctant to answer questions related to the animal numbers involved in the trade, it was not possible to estimate the number of skins being offered for sale for each species recorded. This paper therefore focuses on the morphological identification of the species and not to the number of skins traded.

RESULTS AND DISCUSSION

Traded species

During visits to Bandim market, morphologically identified dried Chimpanzee skins were found being sold for traditional medicinal purposes. Additionally, dried skins from Temminck's Red Colobus monkeys *Procolobus badius temminckii*, Guinea Baboons *Papio papio* and Olive Baboons *Papio anubis* were also found. The authors also detected trade in dried skins of several non-primate species such as Leopard *Panthera pardus*, Nile Crocodile *Crocodylus niloticus*, African Civet *Civettictis civetta*, elephant *Loxodonta* sp., hare *Lepus* sp., African Buffalo *Syncerus caffer*, Spotted Hyaena *Crocuta crocuta* and several species of antelopes, snakes and lizards, as well as skins alleged to be of Wild Dog *Lycaon pictus* and Lion *Panthera leo* (Fig. 1). Other animal body parts observed included bones, Crested Porcupine *Hystrix cristata* spines, teeth, antelope horns, pangolin *Manis* sp. scales, mollusc shells, fish bones and feathers. Morphologically specific identification was not possible in most cases due to the similarity of those body parts to other species, as well as to their condition. A few sellers mentioned that some of the bones being offered for sale were from primates.

All the species mentioned above are reported as occurring in Guinea Bissau except for Olive Baboons, whose western limit of distribution is reported to be in Mali and the Republic of Guinea (IUCN, 2011). The Olive Baboon skin seen was morphologically quite different to the Guinea baboon skins found at the market. While Guinea Baboon skins present red/brownish coloration, the Olive Baboon skin had a green hue, typical of what has been described for the subspecies (Groves, 2001).

Costs, origin and scale

Interviews with urban traders revealed that the cost of a piece of Chimpanzee skin was relatively high, ranging from XOF1500 (CFA Francs) to XOF90 000 (USD2.9 to USD173.96, based on an exchange rate in 2008 of XOF460.77 to USD1). The average monthly wage in 2008 was XOF40 000 (approximately USD88.00) (UNDP, 2010).

All urban vendors reported that the Chimpanzee and other animal body parts (apart from the elephant hide seen) originated from the "southern part", and frequently mentioned the regions of Cantanhez and Gabú specifically. Vendors considered the "southern part" every location south of Bissau. The authors were told that the elephant hide had come from Senegal. According to Blanc *et al.*, 2007, at least one, and at most 10 elephants remain in Senegal. Most vendors said that consumers were of both sexes, different ethnic groups and social status.

It was apparent to the authors that witchdoctors are not the only people to buy animal-derived products for traditional medicine or protection fetishes. For example, according to statements from three vendors:

"All sort of people buy. Men and women, poor or rich... Fulas, Pepel, Balanta, even Europeans. Every kind. Not only djamba kuss [witchdoctors] to please the irans [magical and religious entities]."

According to Robillard, *in litt.* to TRAFFIC, July 2011, it is common practice in Africa for people who are unwell to buy their own products based on a list provided by the traditional doctor. See also Marshall (1998).

Two of the vendors also mentioned that individuals from neighbouring countries such as Senegal, Guinea or Gambia are involved in the trade within the country: “Other foreigners also buy and sell their own plants, shells or skins”.

Symbolic and medicinal use

Most male informants in rural CNP and Boé villages associated the use of Chimpanzee-derived products with the needs of women, as revealed by one elder Fula respondent in Béli, Boé:

“*Dári [chimpanzee] is mezinho [traditional medicine] of women.*”

Three Balanta women in CNP confirmed that Chimpanzee skin is used to: “*prepare a cleansing mixture against hideousness when they are pregnant or their children are still babies in the event they see a lonely chimpanzee cross their way*”. Likewise, another woman said that “*the leaves of the nest where a menstruating female chimpanzee sleeps can be applied to heal mental problems*”.

One informant admitted that he uses a stitched amulet made of chimpanzee body parts to help provide awareness to protect him and his friends while in the bush (Rui Sá, pers. obs., 2008).

Guinea-Bissau in the context of previous studies

One possible explanation for the lack of information on magic practices and traditional medicines using animal body parts in Guinea-Bissau is the difficulty in collecting information on such an undisclosed subject, as well as both a lack of interest and in-depth study of such practices. As a result, the authors’ observations are opportunistic. However, the use of animals’ body parts for medicinal purposes could seriously threaten the biodiversity of Guinea-Bissau and, in particular, constitutes an additional and significant threat to Chimpanzee populations already menaced by habitat loss and fragmentation, the pet trade and crop-raiding conflicts. Therefore, this phenomenon deserves to be thoroughly investigated (Cá, 2008).

Although not previously reported for Guinea-Bissau, the use of non-human primate body parts in traditional medicine is not unusual elsewhere in the world (Alves *et al.*, 2010; Leypey and Fomine, 2010). In a recent review, Alves *et al.* (2010) reported the use of 101 species of primates in folk/magic-religious practices, most frequently in Africa, Latin America and Asia. Although Cercopithecidae species are the most affected, Chimpanzees are also referred to as a remedy for diseases and for use in folk medicine (Alves *et al.*, 2010). In Nigeria, Mali, Sierra Leone, Congo and Guinea, Chimpanzee body parts are used to cure male impotency, epilepsy, bone fractures and infertility in women (Dedeke and Aboyami, 2006). In Cameroon, the Bakweri people believe that by using the liquid derived from boiled Chimpanzee bones, the bones of children or babies will become stronger (Leypey and Fomine, 2010). Additionally, in the forested areas, people use

Chimpanzee body parts in birth and circumcision rituals (Mallart Guimera, 1981). The Yoruba people of south-western Nigeria believe in the magical properties of Chimpanzee body parts in appeasing witches and fortune tellers (Dedeke and Aboyami, 2006). However, it is not easy for people to obtain these remedies or to gain access to these animals. In Central Africa, the consumption of Chimpanzee meat is taboo for young men, pregnant women and children (Robillard, *in litt.* to TRAFFIC, July 2011).

The presence in Bandim market of the skin of an Olive Baboon suggests a foreign origin for some of the animal body parts being offered for sale. While the distribution area for this species (*Papio anubis*) includes neighbouring Guinea and Mali, it does not occur in Guinea-Bissau (Soewu, 2008). The Guinea Baboon *Papio papio* is the only baboon species reported and observed in the country (IUCN, 2011). There are striking differences in morphology between both baboon species (namely coat coloration (Groves, 2001)), which enables a distinction to be made based on their skins. Furthermore, in Colobane and Boucotte markets in Senegal (in Dakar and Ziguinchor, respectively), several species of reptiles and mammals, including primate species (data not shown) were found in trade for use in traditional medicinal practices and/or magic ceremonies (Fernando Sousa, pers. obs., 2008). According to information provided by the sellers, those animal body parts were brought from Niger, Nigeria, Ivory Coast and Mali. Chimpanzee skins were also found in these Senegalese markets (Fernando Sousa, pers. obs., 2008). The respondents pinpointed Cassamance (on the border between Senegal and Guinea-Bissau) as the putative origin of Chimpanzee skins at Boucotte market, and Guinea-Bissau and the Republic of Guinea as the possible origin of the Chimpanzee skins being sold at Colobane market. The possibility that the Chimpanzee skins found in Bandim market could also be from the Republic of Guinea cannot be excluded since sellers mentioned the “south” as the origin but not specifically the south of Guinea-Bissau.

Implications for conservation

The suggested transnational interest for Guinea-Bissau Chimpanzee skins may constitute an even bigger threat for the conservation of this population. Since Chimpanzee populations are declining in West African countries (IUCN, 2010), foreign hunters could be attracted to Guinea-Bissau and the hunting of Chimpanzees could therefore increase in the near future. Biodiversity management authorities in Guinea-Bissau (IBAP and *Direcção Geral de Florestas e Fauna*) have introduced new laws to regulate the trade in wild meat (e.g. recently, the hunting of primates throughout the country was prohibited (Anon., 2011)). However, the lack of resources and lack of awareness of management authorities and politicians is hindering law enforcement in the country. At the international level, conservation agencies should re-examine their strategies to mitigate this trade, and, at the national level, specific programmes should be designed and applied to empower all actors involved (e.g.

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park rangers, Customs officers, the military, police, etc.), complemented at the same time by provision of environmental education for the local communities.

Further work by the authors will include the molecular determination of the origin of the skins observed in the markets and of the species involved. This will assist in evaluating the scale of the trade. Finally, an ethnographic study specifically centred on the use of non-human primate body parts by traditional medicine using more in-depth techniques, such as participant observation or long-term observation, will allow the authors to draw up possible differences in the use of distinct animal parts and determine how such practices are disseminated.

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Chapter 8 Appendix 2 - Optimisation of laboratory procedures

A1.1 – Pilot study

A1.1.1 – Preservation of faecal samples

The method to preserve the faecal samples can affect the amount of DNA extracted (Frantzen *et al.* 1998; Taberlet *et al.* 1999; Roeder *et al.* 2004; Nsubuga *et al.* 2004). The preservation of DNA in faecal samples is achieved by inhibiting the action of nucleases that degrade the DNA until DNA extraction (see review by Beja-Pereira *et al.* 2009).

During the pilot study, I determined which faecal DNA preservation method would be the most successful with respect to the field conditions in Guinea Bissau. Thus, faecal samples were collected and preserved using three different methods: immersed in ethanol 99%, subjected to the two-steps method (as described Roeder *et al.* 2004) and preserved using silica gel only (Type III, S-7625, indicating for desiccation, Sigma-Aldrich ® Company Ltd, Dorset, UK). These methods were chosen over others due to logistical practicability. For example, the preservation of samples at low temperatures was not possible since the field site had no access to electricity and preservation of samples using RNA later would increase the financial costs.

Two extraction methods were tested: the manufacture's protocol for total genomic DNA extraction for human stool of QIAamp® DNA Stool Mini Kit (QIAGEN ®) and an alternative DNA extraction method consisting in a modified protocol from the one published by Flagstad *et al.* (1999). The Flagstad *et al.* (1999)'s DNA extraction modified protocol begins with gently washing of the sample (preserved using silica gel only) with 30ml of PBS (Phosphate buffered saline, 1x) instead of only using the scraped surface of the sample. This pre-washing of the sample surface may have benefits over scraping the surface of the sample since the PCR inhibitors resultant from the animal's diet, (possibility present below the surface of the sample) are not accidentally included in the DNA extraction. The resultant supernatant of this "washing" was centrifuged for 45 minutes and then discarded,

maintaining the epithelial cells in the bottom of the tube. Finally, the ASL Buffer is added to this tube and the extraction follows the QIAGEN ® QIAamp DNA Stool Mini Kits protocol according with the manufacture's manual.

To compare between the storage protocols (two steps, 99% ethanol, desiccation by silica gel) and between “simple” extraction and the alternative “washing” protocol, 10 samples were randomly selected. The quality of amplification was evaluated using a fragment of approximately 490bp of mtDNA (amplified using primers designed and publish by Hapke *et al.* (2001) (Chapter 2, Chapter 4) and a multiplex PCR reaction, in which 3 microsatellite loci are amplified. 2uL of PCR product were subjected to a 2% agarose gels electrophoresis (120V) and then visualized with ethidium bromide in a 3 UV transilluminator (UVP Gel doc it TM, Cambridge, UK). Success of amplification was evaluated by relative brightness of bands in the agarose gel.

Results showed no significant differences between the four procedures for the mtDNA amplification (see fig. 1A). However, for nuclear DNA amplification, the two-steps protocol with “simple” extraction protocol performed consistently better than the other protocols across all the samples tested (see fig. 2A).

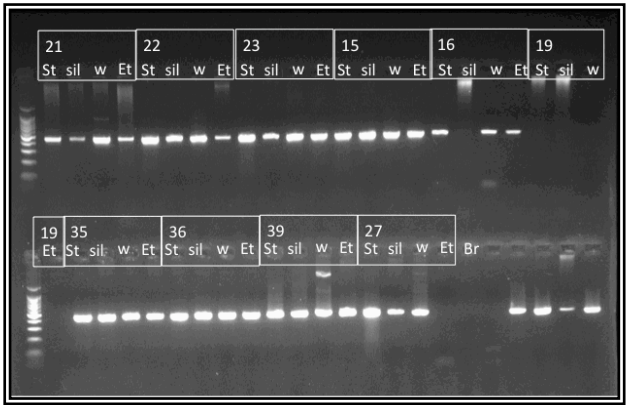


Figure 1A: Comparison of storage methods in the quality of mtDNA amplification (approximately 490bp) (St– two steps; sil – silica gel; w – wash; Et – Ethanol).

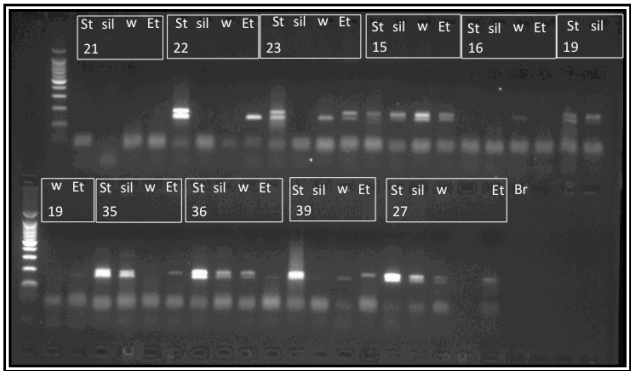


Figure 2A: Comparison of storage methods in the quality of amplification for nuclear DNA (St– two steps; sil – desiccation by silica gel; w – wash; Et – Ethanol). Sample number 22St and 27St shows the right pattern for the multiplex amplification.

The two-step method was chosen to preserve the faecal samples collected in the second sampling phase. The “simple” DNA extraction protocol was further optimised to maximize the amount of DNA extracted (see section 2.2.1).

A.1.2 – Design of Microsatellite PCR Multiplexes and PCRs optimisation

In a multiplex PCR two or more loci are amplified at the same time in the same reaction, reducing the number of PCRs performed per sample and thus reducing time and laboratorial costs (Guichoux *et al.*, 2011). Another advantage of a multiplex PCR is the increased availability of target DNA per locus, reducing the overall quantity of DNA extract used when compared with singleplex PCRs (Beja-Pereira *et al.*, 2009; Guichoux *et al.* 2011). However, in return, a multiplex PCR protocol needs to be optimized carefully to avoid unequal or lack of amplification of some loci across samples with different DNA concentrations (Henegariu *et al.*, 1997), as is often the case for non-invasive DNA samples.

The optimisation protocol for a multiplex PCR starts with the assembling of loci with the same annealing temperature (thus loci amplifiable in the same PCR reaction) and with no primer-dimer interactions between primers (Henegariu *et al.* 1997). After determining the specific allelic range of each locus to be integrated in a multiplex (by amplifying and genotyping separately), it is possible to determine which are the loci with the same annealing temperature and different allele range (and thus labelled with the same fluorescent colour) or have the same allele range size (and therefore should have different fluorescent label). Finally, the concentration of primers of each multiplex is optimized, accounting for preferential locus amplification or non-amplification (Henegariu *et al.*, 1997). At this stage of the multiplex design, samples collected in different populations should be used to assure that all alleles are recognized. This procedure will identify the true population allelic range size or if any locus is monomorphic and also avoiding finding new alleles during the genotyping process that could overlap with the allelic range of other loci included in the same the multiplex (Guichoux, 2011).

For this research, thirteen autosomal and one Y chromosome-linked microsatellite loci, firstly described for humans but with cross-amplification and variable for *Papio* genus were randomly chosen (see list in table 1A). Most of the microsatellite loci used had a

tetranucleotide repeat motif, with the exception of the D7S503 and D13S159. The optimum annealing temperatures of each microsatellite locus were achieved by a gradient PCR using 3 samples. Afterwards, it was determined which loci had compatible annealing temperature and allele range size for integrating a multiplex PCR. For this test it was used 20 samples collected during the first sampling phase. In the end of the process, four multiplexes PCRs were designed (for a detailed description of each multiplex see table 1A) with all primers added to the multiplexes PCR reaction in equimolar amounts. Note that although multiplex 1 and multiplex 4 had the same annealing temperatures and potentially a joint multiplex PCR with six loci could be designed, the quality of the samples hindered such procedure.

The PCRs were carried out using the QIAGEN ® Multiplex PCR Kit in a final volume of 10uL. The final PCRs concentrations used were: 1x QIAGEN Multiplex PCR Master Mix ®, 0,2uM of each primer and 0,75uM BSA. 0,9uL of DNA extract was used initially and then increased according with the lower quality of samples.

All the multiplexes PCR started with a HotStarTaq DNA Polymerase activation step, during 15 min at 95°C, followed by 40 cycles of denaturation step at 94°C for 30 sec, annealing temperature varying between 55°C to 59°C during 30-40 seconds (depending of the multiplex) and extension at 72°C for 40 sec. The PCR ended with a final extension of 30 min at 60°C. PCRs were performed in an AB Applied BiosystemsTM (California, USA) Veriti 96 Well Thermal Cycler.

Appendix

Table 8.1: First loci set used in this Project.

MULTIPLEXES	GENBANK ID F / R PRIMER (5'-3')	REPEAT	RANGE SIZE (BP)	LABEL (5' END-F)	PCR PROFILE
M1	D3S1766 (G08269) ACCACATGAGCCAATTCTGT ACCCAATTATGGTGTGTACC	ATCT	200-192	FAM	95°C for 15min, 40 cycles: 94°C for 30s, 57°C for 40s, 72°C for 40s and 72°C for 30 min.
	D12S375 (G08936) TTGTTGAGGGTCTTTCTCCA TCTTCTATTTGGAAAAGTAACCC	GATA	161-193	HEX	
	D7S503 (G18277) ATGACTTGGAGTAATGGG AACCTTTAATCAGGATACAGAC	CA	142-154	NED	
M2	D1S533 (G07788) CATCCCCCCCCAAAAATATA TTGCTAATCAAATAACAATGGG	GATA	187-195	FAM	95°C for 15min, 40 cycles: 94°C for 30s, 55°C for 40s, 72°C for 40s and 72°C for 30 min.
	D14S306 (G09055) AAAGCTACATCCAAATTAGGTAGG TGACAAAGAACTAAAATGTCCC	GATA	167-183	HEX	
	D4S243 (M87736) AATCCCTTTTCTACCTTTCTATCAC GAGAGGAGAGATAAAAAGATGTAAATG	GATA	150-166	NED	
M3	D21S1442 (G08136) CTCCTCCCCACTGCAGAC TCTCCAGAATCACATGAGCC	GATA	225-241	FAM	95°C for 15min, 40 cycles: 94°C for 30s, 59°C for 50s, 72°C for 40s and 72°C for 30 min.
	D10S611 (G08794) CATACAGGAAACTGTGTAGTGC CTGTATTTATGTGTGTGGATGG	GATA	129-145	FAM	
M4	D3S1768 (G08287) GGTTGCTGCCAAAGATTAGA CACTGTGATTTGCTGTTGGA	GATA	205-221	HEX	95°C for 15min, 40 cycles: 94°C for 30s, 57°C for 40s, 72°C for 40s and 72°C for 30 min.
	D13S159 (Z16691) GCTGTGACTTTTAGGCCAAA TGTGATGTCTACAACTCCAGG	CA	145 (monomorphic)	FAM	
	D5S1457 (G08431) TAGGTTCTGGGCATGTCTGT TGCTTGGCACACTTCAGG	GATA	124-132	HEX	
DYS391	DYS391 (G09613) CTATTCATTCAATCATACACCCA GATTCCTTTGTGGTGGGTCTG	GATA	250 (monomorphic)	FAM	95°C for 15min, 40 cycles: 94°C for 30s, 53°C for 40s, 72°C for 40s and 72°C for 30 min
D5S1470	D5S1470 (G08475) CATGCACAGTGTGTTACTGG TAGGATTTTACTATATCCCCAGG	GATA	150-170	FAM	95°C for 15min, 40 cycles: 94°C for 30s, 50°C for 40s, 72°C for 40s and 72°C for 30 min

Appendix

Although the protocol for the multiplex PCRs was accomplished, this set of thirteen microsatellite loci displayed some problems. Due to their unique annealing temperatures, two loci could not be multiplexed (D5S1470 and the DYS391) and two loci were monomorphic (i.e. amplifying the same allele for all samples) (D13S159 and DYS391). After removing the monomorphic loci and the locus that could not be multiplexed, the set of eleven microsatellite loci proved to have a low probability of identity (PI) (the probability that two individuals share the same genotype, Waits *et al.* 2001), which could increase the probability of excluding different individuals assuming they were the same individual. The total probability of identity using the eleven loci (the probability that two individuals share the same genotype, P_{Ibiased}) was $1,11 \times 10^{-01}$ (or using the more conservative measure for populations with related individuals $PI_{\text{sibs}} = 6,87 \times 10^{-1}$). In table 2A, the eleven microsatellite loci set are ranked according to its respective PI: the locus D21S1442 is the best to distinguish between individuals ($PI_{\text{biased}} = 9,87 \times 10^{-02}$ and $PI_{\text{sibs}} = 3,96 \times 10^{-01}$) while the D5S1457, D7S503, D10S611 and D13S159 are the loci with less ability of distinction (see table 2). According with Waits *et al.* (2001), a PI level of at least 0.01 should be used in the case of molecular identification of endangered species (which are expected to show higher values of inbreeding), which is not accomplished with the set of loci used (Table 2).

Table 8.2: PI of loci

Locus	PI_{biased}	PI_{biased}(cum)	PI_{sibs}	PI_{sibs}
D21S1442	9,87E-02	9,87E-02	3,96E-01	3,96E-01
D14S306	1,05E-02	1,09E-01	1,60E-01	5,56E-01
D4S243	1,19E-03	1,10E-01	6,62E-02	6,22E-01
D3S1766	2,39E-04	1,11E-01	3,17E-02	6,54E-01
D12S375	4,44E-05	1,11E-01	1,54E-02	6,69E-01
D1S533	9,72E-06	1,11E-01	7,58E-03	6,77E-01
D3S1768	2,92E-06	1,11E-01	4,17E-03	6,81E-01
D5S1457	9,19E-07	1,11E-01	2,42E-03	6,83E-01
D7S503	3,28E-07	1,11E-01	1,47E-03	6,85E-01
D10S611	1,39E-07	1,11E-01	9,86E-04	6,86E-01
D13S159	1,26E-07	1,11E-01	9,39E-04	6,87E-01

As a consequence, these four PCR multiplexes were re-designed with the inclusion of five new loci (D13S765; D2S1326; D7S2204; D8S1106; D6S501), which was carried out by Gisela Fickenscher (German Primate Center) as a part of a collaboration established in January 2010. Gisela Fickenscher used the PCRs conditions of the initial four multiplexes, included the new loci and optimized the primers concentrations in the reactions.

The end-fluorescent primer's labels were changed from Gisela's final multiplex PCR protocol from 3-dye system (6-FAMTM, HEXTM and NEDTM, three colours: blue, green and black) using 400HDTM size standard system (Fickenscher, 2010) to a 4-dye system using 500 LIZTM size standard system in multiplex 1,4,3 and 5. The 500 LIZ® size standard system enables the use of four different dyes (four different colours): 6-FAMTM (blue), VICTM (green), NEDTM (black) and PETTM (red) (Applied Biosystems) allowing for a greater number of loci to be analysed together and thus decreasing the laboratorial costs (see Fig. 3A). In addition, the locus D4S243 was amplified in a singleplex PCR (instead of being included in Gisela's multiplex 5, Fickenscher 2010). Finally, the genetic marker for the sex molecular determination protocol was included in multiplex 5 (the forward primer was end-labelled with PET fluorescence, Applied Biosystems) instead of determining the sex by agarose electrophoresis (Fickenscher 2010).

Chapter 9 Appendix 3 – Supplementary material for Chapter 3

Table 1: Samples collected at the markets and identified using a Barcoding approach

Species	N individuals observed			N tissue samples collected			% Tissue samples collected per individuals	N tissue samples labelled and	Error rate (%)	Tissue samples assigned to sp.
	Chapa	Rampa	Total	Chapa	Rampa	Total				
<i>Cercopithecus campbelli</i>	70	21	91	19	3	22	22.7	9	59.1	11
<i>Erythrocebus patas</i>	1	3	4	1	0	1	25	1	0	2
<i>Procolobus badius</i>	18	4	22	10	0	10	45.5	6	40	7
<i>Colobus polykomos</i>	3	0	3	3	0	3	100	3	33.3	3
<i>Papio hamadryas papio</i>	18	8	26	12	0	13	50	12	7.7	12
<i>Chlorocebus sabaeus</i>	3	1	4	1	0	1	25	1	0	15
Total	113	37	150	46	3	50				

Table 2: Calculations of the true positive identification frequency (A) and the false negative identification frequency (B)

Species	A				B				
	NM (Morphological records)	TL (Tissue samples labelled and assigned to each sp)	TS (Tissue samples collected per sp.)	A	TNL (Tissue samples assigned to specie <i>minus</i> Tissue samples labelled and assigned to the species)	NSOS (Tissue samples collected for all species <i>minus</i> Tissue samples collected for each species)	IM (Morphological records for all species <i>minus</i> Morphological records for each species)	B	A+B
<i>Cercopithecus campbelli</i>	91	9	22	37.2	$(11 - 9) = 2$	$(50 - 22) = 28$	$(150 - 91) = 59$	4.2	41.4
<i>Papio hamadryas papio</i>	26	12	13	24.0	1	37	124	3.4	27.4
<i>Chlorocebus sabaeus</i>	4	1	1	4.0	14	49	146	41.7	45.7
<i>Erythrocebus patas</i>	4	1	1	4.0	1	49	146	3.0	7
<i>Colobus polykomos</i>	22	6	10	13.2	1	40	128	3.2	16.4
<i>Procolobus badius</i>	3	2	3	2.0	0	47	147	0.0	2.0

Example of calculations (For Mona monkeys, *Cercopithecus campbelli*):

Calculation of A

NM (number of morphological records) = 91

TL (Number of tissue samples identified morphologically as mona monkeys and molecularly assigned as mona monkeys) = 1

TS (Number of tissue samples collected for that species as identified by the sellers) = 22

Therefore: $A = 91 \times (1/22) = 37.23$

Calculation of B

IM (Number of individuals in both markets which were not morphologically identified by the sellers as mona monkeys) = $150 - 91 = 59$

TNL (Tissue samples molecularly assigned to mona monkeys but not identified morphologically by the sellers as that species) = $11 - 9 = 2$

NSOS (Number of tissue samples collected at both markets that were morphologically identified by the sellers as mona monkeys) = $50 - 22 = 28$

Therefore: $B = 59 \times (2/28) = 4.2$

$A + B = 37.23 + 4.2 = 41.4$

Table Appendix 5 2: Primer sets used in the molecular identification

Primers sets	Forward sequence Reverse sequence	Fragment size	Species sequenced
OWMCOI	(A/G)CT (G/C)TT TTC AAC AAA (C/T)CA (C/T)AA AGA C GTA (A/G)AC TTC (G/C)GG GTG (A/G)CC (A/G)AAG AA TC	~700bp	<i>Papio papio</i> , <i>Chlorocebus sabaeus</i> , <i>Erythrocebus patas</i> , <i>Cercopithecus</i> <i>campbelli</i> , <i>Procolobus badius</i>
VERTCOI	TTC TCA ACC AAC CAA CAA AGA CAT TGG TAG ACT TCT GGG TGG CCA AAG AAT CA	~700bp	<i>Procolobus badius</i> (1 sample) <i>Cercopithecus campbelli</i>
FOLMER	GGT CAA CAA ATC ATA AAG ATA TTG G TAA ACT TCA GGG TGA CCA AAA AAT CA	~700bp	<i>Colobus polykomos</i>

Chapter 10 Appendix 4 - Using genetics as a tool in primate conservation

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Using Genetics as a Tool in Primate Conservation

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"Here I am, in the middle of this forest, and I can't see the primates? How can I learn more about the species I am studying?" This question can be answered with the help of recent advances in non-invasive molecular genetics.



The current conservation status and decline of nonhuman primates is alarming (Norconk *et al.* 2011) due to factors such as **disease**, **habitat** destruction, hunting, illegal trade and **climate change** (WWF 2010). Unfortunately, their restricted geographical range, resource requirements, long lifespan and slow **reproduction** rate, **dispersal** needs, and degree of specialization, make primates more susceptible to **extinction** than many other **species** (Cowlishaw & Dunbar 2000, Harcourt *et al.* 2002, Gibbons & Harcourt 2009). Moreover, the geographical range of threatened primate species often overlaps with areas of high human density (Harcourt & Parks 2003). Recent studies conclude that 48% of primate species are in danger of becoming extinct, making well-informed conservation measures crucial for ensuring their long-term survival (Mittermeier *et al.* 2009). Aside from their contribution as models for human evolutionary research, their value to **ecotourism**, and their charisma, primate species play important ecological roles, especially as seed dispersers (Chapman & Russo 2007). Some studies suggest that maintaining this role could be important for ecosystem resilience because monkeys and apes visit trees in **social groups** where they tend to stay longer during feeding periods than other mammals or birds (Lambert 2011).



Figure 1: Elusive animals.

Guinea baboons (*Papio hamadryas papio*) live in dense forested habitats and are very hard to observe. By using molecular tools, primatologists can gather information about this elusive species.

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Non-Invasive Genetic Analysis

The relatively recent **development** of non-invasive genetics has allowed primatologists to better understand the **population** and group dynamics of wild primates, simply by the fact that it is now possible to obtain genetic information by extracting **DNA** from by-products such as feces, shed hair, and urine. The first study of this kind was in chimpanzees (*Pan troglodytes*) in which Morin *et al.* (1993) analyzed patterns of **gene flow** in the Gombe chimpanzee **community** (Tanzania). Since then, major technical improvements in non-invasive genetics have greatly expanded our capacity to address a wide range of questions about the structure of primate populations, their evolutionary histories, and **adaptation**, while allowing the study of wild populations without direct contact with the animals (Charpentier *et al.* 2007, Tung *et al.* 2008). More importantly, the combination of genetics with long-term socio-ecological data has enabled comprehensive analysis at an individual and social group level for a wide range of primate species.

DNA obtained non-invasively can be analyzed in individuals and populations for a wide range of molecular genetic markers such as **microsatellites**, **minisatellites**, **mitochondrial DNA**, **amplified fragment length polymorphism (AFLP)** and the **major histocompatibility complex (MHC)**. By using a variety of software to analyze the genetic data produced, primatologists can now obtain information on **effective population size**, **parentage**, **relatedness**, **sex**, **dispersal**, **population structure**, **population assignment** and **gene flow**. This is crucial if we are to fully understand population dynamics at a local **scale** and evaluate the threats and suggest appropriate conservation measures (Goossens & Bruford 2009).

Threats to Primate Conservation

Habitat fragmentation

Habitat fragmentation can influence several key features of primate populations: 1) their diet, 2) the social group size or density, and 3) the dispersal and gene flow between social groups or subpopulations (Marsh 2003, Frankham *et al.* 2002). Consequently, the capacity for populations to persist in fragmented landscapes is related to a species' particular characteristics (Marsh 2003) (Figure 2). It is theoretically possible for primate populations to increase in size within fragments—for example, if natural predators disappear (Strier 2007)—but more frequently, they decrease or become extinct. This can be due either to direct mortality, caused by an increased hunting pressure, since isolated areas become accessible for humans (Marsh 2003, Strier 2007), or due to genetic changes (Frankham *et al.* 2002) (Figure 3). In the long term, **fragmentation** can lead to a reduction in genetic diversity and increased genetic **differentiation**. This results from the decrease of gene flow between breeding groups and the action of random **genetic drift** and/or **inbreeding** (Frankham *et al.* 2002).



Figure 2: Behavioral adaptation to habitat fragmentation.

Black-and-white colobus are almost exclusively arboreal. Fragmentation may force the adaptation to new habitats or result in local extinctions.

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Figure 3: Habitat loss and deforestation.

Deforestation is happening at an accelerated rate and can be caused by logging, collection of non-timber forest products, and fires. It not only promotes isolation of populations but also increases hunting pressure and contributes to climate change.

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Each population fragment may show different levels of genetic diversity and significantly different **allele** frequencies from the other fragments. The risk of **inbreeding depression** is increased if the population is smaller and isolated, with lower genetic diversity.

Appendix

Migration of individuals between fragments and subsequent reproduction will introduce new **alleles** into the population (increasing genetic diversity) and it will counterbalance the effects of genetic drift and inbreeding, preventing complete **fixation** of alleles (Frankham *et al.* 2002). By using non-invasive genetic methods it is possible to identify the genetic structure of a fragmented population, and levels of gene flow between units, and determine whether ecological **corridors** should be created/maintained, or individuals should be translocated (e.g., Bruford *et al.* 2010).

In the fragmented range of the Cross River Gorilla (*Gorilla gorilla diehli*) three sub-populations have been uncovered using **microsatellite** markers (Bergl & Vigilant 2007). Although this genetic structure corresponds broadly to the pattern of habitat fragmentation, migrants between fragments could be identified. Since different levels of genetic diversity were found between the sub-populations, it was suggested that the conservation of the most genetically diverse sub-population should be prioritized. Also, habitat corridors between fragments, along with measures to control hunting in areas between fragments, were recommended (Bergl *et al.* 2008). The Bornean orang-utan (*Pongo pymaeus*), living in forest fragments of the Lower Kinabatangan flood plain in Sabah, Malaysia, shows a different pattern: high levels of **heterozygosity** within fragments, with a relative scarcity of rare alleles, suggesting that this population was large in the past and has suffered a recent major reduction (Goossens *et al.* 2005). Goossens *et al.* (2006), using extensive non-invasive sampling across the area and 14 microsatellite loci, showed that the Bornean orang-utans population has decreased in size by 95% over the last decades or centuries, due to anthropogenetic fragmentation of the habitat. Therefore, the high genetic diversity found is transitory and may disappear if forest corridors alongside the riverbank are not established (Bruford *et al.* 2010).

Hunting

The impact of hunting pressure on primate populations is often difficult to evaluate. Although information on the amount of harvested primates can be obtained by counting carcasses in urban bushmeat markets, morphological identification can be hindered if a carcass has been processed, or if the meat has been smoked (Figure 4).



Figure 4: Bushmeat markets. Hunting of primates is occurring at very high rates. Primate meat is consumed in rural areas for subsistence and in urban centers as a delicacy. It is the result of an illegal organized trade.

Appendix

Primatologists can use molecular PCR-based tools to taxonomically identify unknown specimens. After extracting DNA and amplifying a specific DNA fragment, these fragments can then be compared with other DNA fragments obtained from specimens of known species. The comparison can also be accomplished by verifying the presence/size of the fragment after PCR: for a review of the techniques see Fajardo *et al.* (2010).

In many cases however, the researcher might not have access to specimens of known species. To overcome this difficulty, it is necessary to amplify a standard gene fragment that can be compared with fragments from voucher species deposited in public databases. A fragment of 648bp from the mitochondrial cytochrome c oxidase (COI) gene was proposed by Hebert *et al.* (2003) as a standard fragment for **DNA barcoding**, the data for which are deposited in the Barcode of Life Database (<http://www.barcodeoflife.org/>) as well as in public databases such as GenBank. Lorenz *et al.* (2005) tested the use of this mitochondrial DNA region to identify the species of primate samples. All samples, representing 56 primate species, amplified with at least one of the 3 different primers used and, with few exceptions, the fragments obtained clustered together with sequences retrieved from GenBank (Figure 5). More recently, Rönn *et al.* (2009) proposed the use of a micro-array system to assign samples of primates to the genus level, using both nuclear and mitochondrial **genes**. This technique uses 111 diagnostic **nucleotide** positions to perform a hierarchical assignment of samples. This method can be used to process a large number of samples at a relatively low **cost**, and 45 out of the 64 samples were correctly assigned to their Primates genus.

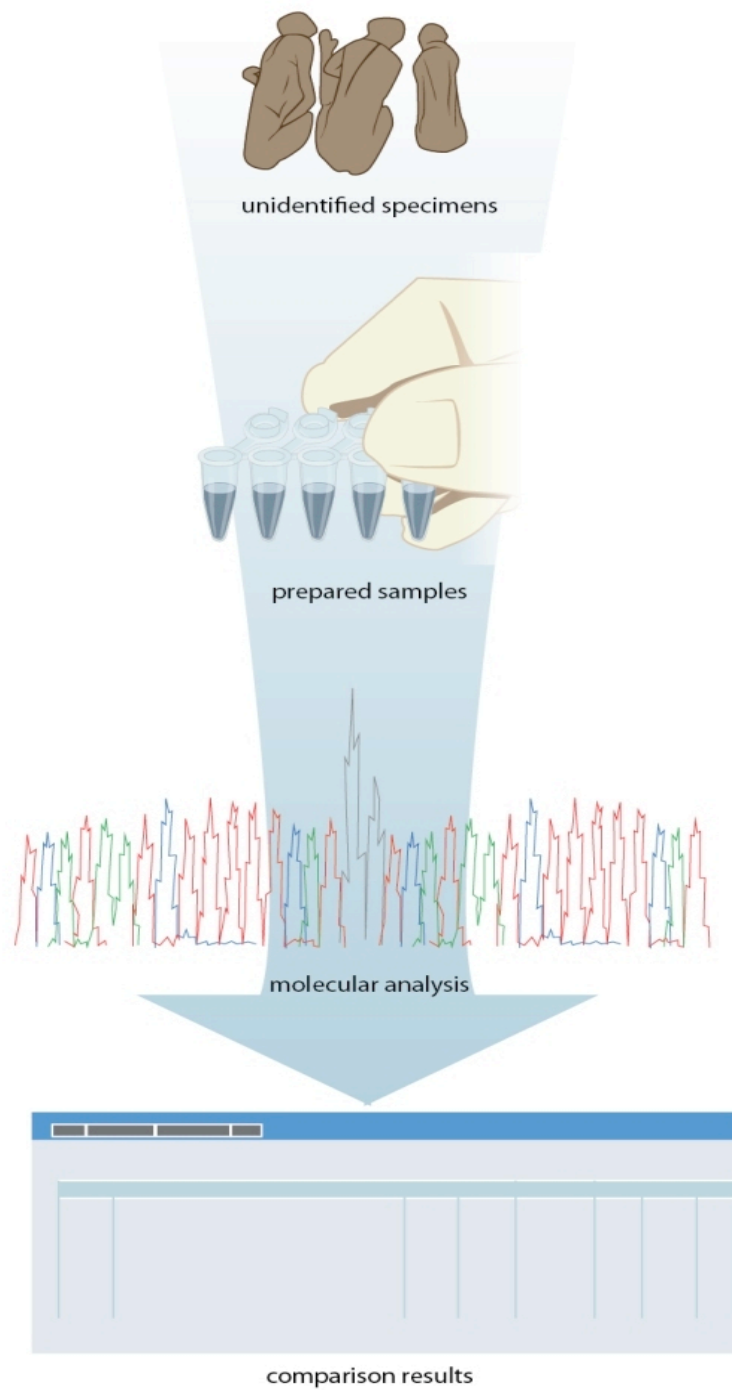


Figure 5: Molecular identification of bushmeat.

Morphological identification can be difficult if carcasses have been processed. Molecular identification is the easiest and most reliable tool available.

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Diseases

Disease is another important **aspect** for primate conservation. The Ebola and anthrax outbreaks that have occurred in Central Africa in recent decades caused a dramatic decline in gorilla and chimpanzee populations (Leendertz *et al.* 2006, Bermejo *et al.* 2007, Campbell *et al.* 2008). Additionally, recent studies on parasite infection dynamics have demonstrated an association with hunting, human population growth, and fragmentation in wild primates (Gillespie & Chapman 2006, Goldberg *et al.* 2007, Gillespie *et al.* 2008, Riley & Fuentes 2011). With the incorporation of molecular approaches to **epidemiology**, Johnston *et al.* (2010) have demonstrated cross species transmission of *Giardia duodenalis* between humans, livestock, and wild primates in Western Uganda. Likewise, Goldberg *et al.* (2009) discovered three novel retroviruses in red colobus monkeys, shedding light on the dynamics of primate retroviral transmission. More recently, Yildirim *et al.* (2010) unveiled the gut microbial community of three nonhuman primate species by sequencing the small subunit **rRNA** unit from fecal samples, allowing future analysis on comparative and evolutionary studies of human gut microbes and other primates. Furthermore, using an innovative method that combines a single-genome amplification of *Plasmodium* sp. recovered non-invasively from fecal material of great apes, Liu *et al.* (2010) inferred that the **origin** of the human malignant malaria *Plasmodium falciparum* is gorilla-derived. This result argues against the previous study from Prugnolle *et al.* (2009) that showed that *P. falciparum* emerged from *P. reichenowi* by a single transfer from chimpanzees. Similarly, HIV/AIDS is the result of a cross-species transmission event of simian immunodeficiency **virus** (SIV) to humans from non-human African primates, and much attention has been paid to the understanding of the evolutionary history of these emerging infection diseases (Gao *et al.* 1999, Damond *et al.* 2004, Liu *et al.* 2008). By using a molecular dating technique, Wertheim and Worobey (2009) estimated a surprisingly recent common ancestor of infectious SIV in chimpanzees (between 1266 to 1685 years ago) and sooty mangabeys (between 1729 to 1875 years ago), the reservoirs of HIV-1 and HIV-2, respectively. Conversely, human transmitted **pathogens** to great apes such as **bacteria** (e.g., *Streptococcus pneumoniae*) or viruses (e.g., human metapneumovirus) are causing fatal respiratory outbreaks (Chi *et al.* 2007, Kaur *et al.* 2008, Köndgen *et al.* 2008, 2011, Palacios *et al.* 2011) and to mitigate the risk of disease transmission the use of face masks by researchers, tourists and staff is advocated as a good practice (Macfie & Williamson 2010). These studies emphasize the fact that there is much to be learned concerning disease transmission and its implications for wild primates using molecular tools.

**Figure 6: Primate parasites.**

a) *Entamoeba* sp. and b) *Strongylida* fam. gen. sp. (Hookworms) are gastrointestinal parasites that can cause

severe infections and weaken the health of the host.

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Applying Conservation Genetics

Primate census

The abundance and density of wild primate populations are key parameters for assessing their conservation status and management (Arandjelovic *et al.* 2010). Biomonitoring and molecular censusing allows the **determination** of population size, as well as individual movements in the landscape (Storfer *et al.* 2007, Vigilant & Guschanski 2009). Guschanski *et al.* (2009), using a panel of 16 microsatellite loci, estimated that the population size of the endangered mountain gorillas (*Gorilla beringei beringei*) was 10% less when compared to the classical nest-count methods. All molecular census estimates in primates have shown a population size smaller than previously accessed by traditional methods. In contrast, Zhan *et al.* (2006), comparing the numbers of traditional survey methods with molecular censusing, demonstrated that the DNA-based estimate for a well-studied giant panda (*Ailuropoda melanoleuca*) population was more than double the ecological estimate. When using a capture-recapture analysis for census purposes, Arandjelovic *et al.* (2010) recommended that three times more samples should be collected than the predicted population size for apes when assuming a closed population **model**. Therefore, molecular surveys provide a **complementary** method to more traditional census approaches.

"Evolutionary significant units" (ESUs) and "management units" (MUs)

ESUs and MUs are two types of conservation units described using genetic information: ESUs have been defined as needing to be reciprocally **monophyletic** mitochondrial lineages (i.e., occupying different branches in a **phylogenetic tree**) and requires long-term historical **population differentiation**, whereas MUs are identified based on current **demographic** isolation (i.e., no current or recent gene-flow), evidenced by differences in **allele frequency** distributions and significantly different frequencies for both mitochondrial and nuclear **loci** (Moritz 1994). Although the criteria to identify these units have been subject to debate (e.g., see Paektau 1999), such definitions can be key indicators to preserve genetic distinctiveness (evolutionary heritage, genetic diversity and differentiation). For example, Kanthaswamy *et al.* (2006) suggested that the Bornean and Sumatran orangutans should be considered two distinct MUs based on the analysis of mtDNA and microsatellite loci, and consequently the authors advised against the inter-island **translocation** of animals.

Population and habitat viability analysis

Population and habitat **viability** analysis (PHVA) evaluates the risk of extinction within a certain period of time (e.g., 100 or 200 years) and identifies which factors play a major role in the extinction process. PHVA relies on **stochastic** modeling by using simulation software, such as VORTEX (Miller & Lacy 2005), and requires the input of parameters on the ecology and life history of the species (e.g., population size, mortality and birth rates, **sex ratio**, dispersal rates, and main threats to the habitat) to be able to simulate (by Monte Carlo iterations) species responses that are realistic. Molecular census and genetic data can also be very important parameters for PHVA. Moreover, it allows the introduction of different and combined management measures (e.g., ecological corridors, reintroduction, translocations, habitat rehabilitation) and simulates the **evolution** of the species under such interventions. This tool allows conservationists to detect the major threats for rare and **endangered species** and thereby help implement the most long-term viable conservation actions.

Bruford *et al.* (2010) incorporated the genetic data of 200 orangutans from the Kinabatangan floodplain in Sabah, Malaysia, to study the implications of non-intervention, translocation, corridor establishment, and a combination of the latter two measures, on the future genetic diversity of this highly fragmented population. They found that non-intervention would result in the extinction of some of the subpopulations within five generations, and that translocation or corridor establishment alone would not be sufficient to prevent high levels of inbreeding. Instead, a combination of the two measures would retain the demographic **stability** of even the most isolated subpopulations and constrain localized inbreeding to a sustainable threshold (Figure 7).

Appendix

	Carrying capacity	Mean (r)	SD	P (E) after 25 / 100 / 250 yr	Initial / mean final pop size	SD	Retained GD after 25 / 100 / 250 yr	Mean F after 25 / 100 / 250 yr	Mean TE
<i>Without inbreeding depression</i>									
PSU 1	230	-0.001	0.037	0 / 0 / 0	230 / 157.7	27.33	0.994 / 0.981 / 0.954	0 / 0.010 / 0.036	0
PSU 2	351	0.001	0.035	0 / 0 / 0	209 / 245.02	39.15	0.994 / 0.985 / 0.967	0 / 0.009 / 0.023	0
PSU 3	108	0	0.045	0 / 0 / 0.001	63 / 71.48	17.24	0.980 / 0.949 / 0.889	0.001 / 0.030 / 0.098	0
PSU 4	72	-0.001	0.051	0 / 0 / 0.017	61 / 44.36	14.1	0.977 / 0.933 / 0.837	0.001 / 0.037 / 0.131	192.2
PSU 5	313	-0.001	0.036	0 / 0 / 0	293 / 218.63	36.51	0.995 / 0.986 / 0.966	0 / 0.008 / 0.027	0
PSU 6	62	-0.002	0.054	0 / 0.029 / 0.036	55 / 37.72	12.52	0.976 / 0.923 / 0.808	0.001 / 0.041 / 0.150	187.8
PSU 7	54	0	0.063	0 / 0.019 / 0.122	23 / 30.93	11.55	0.947 / 0.964 / 0.732	0.003 / 0.080 / 0.216	149.5
PSU 8	97	0.003	0.056	0 / 0.022 / 0.076	22 / 58.41	19.12	0.946 / 0.878 / 0.796	0.003 / 0.079 / 0.174	137.5
PSU 9	49	-0.003	0.06	0 / 0.006 / 0.079	49 / 28.45	10.91	0.971 / 0.900 / 0.760	0.001 / 0.052 / 0.204	185.2
PSU 10	31	-0.003	0.075	0 / 0.069 / 0.349	23 / 17.13	7.48	0.944 / 0.827 / 0.628	0.002 / 0.089 / 0.299	152.8
PSU 11	120	-0.001	0.043	0 / 0 / 0	97 / 79.02	19.33	0.987 / 0.966 / 0.906	0.001 / 0.021 / 0.076	0
<i>Including inbreeding depression</i>									
PSU 1	230	-0.002	0.037	0 / 0 / 0	230 / 144.39	30.32	0.994 / 0.981 / 0.952	0 / 0.009 / 0.036	0
PSU 2	351	0	0.035	0 / 0 / 0	209 / 227.16	39.45	0.994 / 0.985 / 0.966	0 / 0.008 / 0.026	0
PSU 3	108	-0.001	0.046	0 / 0 / 0.016	63 / 52.84	19.55	0.980 / 0.949 / 0.874	0.001 / 0.025 / 0.097	216
PSU 4	72	-0.004	0.054	0 / 0.001 / 0.068	61 / 30.43	14.61	0.979 / 0.933 / 0.808	0.001 / 0.030 / 0.133	198.1
PSU 5	313	-0.001	0.036	0 / 0 / 0	293 / 203.83	38.07	1.000 / 0.986 / 0.965	0 / 0.006 / 0.026	0
PSU 6	62	-0.005	0.058	0 / 0.005 / 0.123	55 / 24.37	12.16	0.976 / 0.923 / 0.780	0.001 / 0.039 / 0.155	197.4
PSU 7	54	-0.004	0.069	0 / 0.032 / 0.388	23 / 18.97	10.53	0.947 / 0.963 / 0.702	0.002 / 0.069 / 0.213	173.8
PSU 8	97	-0.002	0.063	0 / 0.038 / 0.275	22 / 33.37	19.31	0.946 / 0.872 / 0.762	0.002 / 0.065 / 0.161	167.4
PSU 9	49	-0.006	0.065	0 / 0.008 / 0.274	49 / 18.1	10.17	0.971 / 0.900 / 0.726	0.001 / 0.050 / 0.189	194.1
PSU 10	31	-0.008	0.08	0 / 0.091 / 0.706	23 / 10.16	6.37	0.944 / 0.874 / 0.611	0.002 / 0.080 / 0.288	164.2
PSU 11	120	-0.002	0.043	0 / 0 / 0.004	97 / 64.03	20.89	0.987 / 0.966 / 0.897	0.002 / 0.020 / 0.076	203.8

Figure 7: Population viability analysis.

The table shows the result of demographic and genetic parameters of the vortex simulation for 25 to 250 years under a non-intervention scenario. Note the mean extinction time (column TE) for most of the sub-populations (PSU) is less than 250 years. P(E): probability of extinction; Pop: population; GD: genetic diversity (from Bruford *et al.* 2010).

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Summary

The extensive use of molecular techniques as tools has provided new opportunities to better understand the mechanisms underlying the evolution and adaptation of primates (Figure 8). By integrating genetic and ecological data into simulation models, conservation predictions will be more accurate, and long-term conservation strategies will be more effective.



Figure 8: Survival of endangered species.

Interspecific association between western Red and Black-and-white colobus (Cantanhez National Park, Guinea Bissau). Prospects for the survival of these endangered species will benefit from the use of non-invasive genetic tools.

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