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Genetic portrayal of two colobine monkeys inhabiting a continuous forest in Gola Rainforest National Park

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Live as if you were to die tomorrow. Learn as if you were to live forever.

— Mahatma Gandhi

Abstract

With most of the human population growth occurring in areas of high biodiversity, it is urgent and crucial to understand and assess the impacts of anthropogenic activity on wildlife. This includes the case of West Africa, a region characterized by a highly anthropogenic landscape, yet home to many threatened non-human primates. In this study, the focus is directed to scanning for possible connections between human presence/activity and patterns of dispersal, genetic diversity, demographic history, and genetic and geographic population structure of two sympatric species of colobus monkeys. Fieldwork was conducted in 2018, using a non-invasive sampling method to obtain fecal matter of the arboreal primates, extant in the Gola Rainforest National Park (GRNP), Sierra Leone. A total of 14 microsatellites for 146 samples of *Piliocolobus badius badius* (Bay colobus) and 15 microsatellites for 25 samples of *Colobus polykomos* (King colobus) were analyzed. Both colobines presented genetically diverse populations, with overall expectable patterns of sex-biased dispersal. The populations were historically large, having seemingly suffered demographic collapses at different phases of the Holocene epoch, possibly due to bioclimatic changes. Neither species appeared to have a strong genetic substructure, although *C. polykomos* presented some substructure at the landscape level. Thus, the results of this study suggest that both species seem to be resilient to fairly recent anthropogenic pressures in this protected area. Since these arboreal primates are highly dependent on the forests for habitat, their genetic status in the GRNP reflects the high level of integrity of the protected area. The findings in this study illustrate the importance of maintaining continuous forest habitat to conserve these primates. The former may be used to inform conservation planning of the international Gola landscape, with the involvement of stakeholders.

Keywords: arboreal primates / colobus monkeys / conservation genetics / landscape genetics / anthropogenic impacts

Resumo

Devido à sobreposição geográfica entre o crescimento demográfico humano e níveis excecionais de biodiversidade, o estudo dos impactos antropogénicos sobre espécies selvagens torna-se cada vez mais relevante e urgente. Compreender estas dinâmicas torna-se especialmente premente na região da África Ocidental, uma região considerada um hotspot de biodiversidade e onde a população humana duplicou na última década. Este território caracteriza-se por uma paisagem extensivamente alterada pelos humanos, onde habitam simultaneamente várias espécies de primatas não-humanos. Neste contexto, é imperativo compreender possíveis conexões entre a presença e/ou atividade humana e os impactos que estas podem ter nas populações de primatas não-humanos, especialmente aqueles mais dependentes da floresta. Este é precisamente o caso dos macacos cólobos africanos, que são espécies arborícolas, com dieta maioritariamente folívora e necessidade de áreas alargadas de floresta para cumprir os seus requisitos alimentares e de dispersão. Devido a estas características, estes animais são particularmente afetados quando os seus habitats naturais são perturbados devido à ação humana, nomeadamente através da destruição do habitat e caça para consumo da sua carne. Este estudo foca-se em duas espécies de macacos cólobos da África Ocidental, o cólobo preto-e-branco (*Colobus polykomos*) e o cólobo vermelho-de-bay (*Piliocolobus badius badius*). Estes primatas arborícolas encontram-se frequentemente em simpatria e partilham as mesmas necessidades ecológicas, apesar de demonstrarem sistemas sociais contrastantes. Tendo em conta o declínio populacional global no caso de ambas as espécies, é essencial a monitorização das suas populações, principalmente devido à sua sensibilidade a distúrbios no habitat. Estes primatas existem no Gola Rainforest National Park (GRNP), que se encontra na região Sudeste da Serra Leoa. O parque inclui três blocos de floresta contínua e protegida, onde a exploração por parte das comunidades locais está interdita. Estas comunidades distribuem-se à volta da área de floresta protegida e podem explorar a zona tampão que a rodeia.

Em 2018, no contexto do projeto PRIMATOMICS, recolheram-se amostras fecais e respetivas localizações de vários grupos sociais das duas espécies nas diferentes regiões do parque. Seguidamente, os dados genéticos foram produzidos pela mesma equipa no laboratório do Instituto Gulbenkian da Ciência, Portugal. Inicialmente, foram analisados 15 microssatélites de 54 amostras do cólobo preto-e-branco e de 198 amostras do cólobo vermelho-de-bay. Calculou-se o Índice de Qualidade (IQ) para cada amostra e *locus*, permitindo a seleção de *loci* e amostras a descartar por não cumprirem os requisitos mínimos de qualidade. Estimaram-se os erros mais frequentes associados à genotipagem ("false allele" e "allelic dropout"), assim como o número mínimo necessário de repetições PCR por *locus* para a obtenção do nível mais elevado de confiança nos genótipos. Verificaram-se também amostras anormalmente diferentes do resto da população usando uma análise de componentes fatoriais. Nos resultados destas verificações de qualidade, detetaram-se três *loci* na população de cólobo preto-e-branco e dois *loci* na de cólobo vermelho-de-bay com mais erros e desvios que esperado. Após a otimização dos dados, foram mantidos 14 microssatélites de 146 amostras de cólobo vermelho-de-bay e 15 microssatélites de 25 amostras de cólobo preto-e-branco para a realização das análises subsequentes. Além disso, criaram-se mais duas bases de dados com os *loci* problemáticos removidos (restando 12 *loci* para cada população), repetindo as mesmas análises. Assim, a comparação dos resultados das análises com as bases de dados completas e com as bases de dados excluindo esses dois *loci* ajudaria à decisão da sua remoção permanente para obter resultados imparciais.

A diversidade genética foi estimada a partir de parâmetros frequentemente usados noutros estudos, incluindo o número total de alelos (N_a), número efetivo de alelos (N_e), heterozigotia esperada (H_e) e observada (H_o), riqueza alélica (A_r) e coeficiente de endogamia (F_{is}). Simultaneamente, foram realizados testes de desvio do Equilíbrio de Hardy-Weinberg (HWE) e presença de Linkage Disequilibrium (LD). Seguidamente, estimaram-se os níveis de parentesco, verificando a existência de um grupo de indivíduos altamente aparentados na população de cólobos pretos-e-brancos. Subsequentemente, inferiu-se a estrutura genética de ambas as populações, confirmando-se a existência de uma subestrutura populacional referente a um grupo familiar de cólobos pretos-e-brancos. A identificação desta família levou a mais uma separação de amostras,

criando-se uma base de dados desta espécie com 18 amostras, excluindo esse grupo. Assim sendo, as análises subsequentes seriam realizadas com, além das quatro bases de dados anteriores, com mais uma base de dados sem o grupo de indivíduos altamente aparentados de cólobo preto-e-branco. Seguidamente, foi realizada uma análise de estrutura genética para estimar do número de grupos genéticos (K), bem como uma verificação destes resultados através do cálculo de Δ K e Posterior Probability. A esta juntou-se uma análise de estrutura de natureza exploratória – Principal Component Analysis (PCA) – e uma verificação do padrão de isolamento por distância dos indivíduos (IBD). A esta juntou-se uma análise de estrutura genética considerando a localização das amostras. Para esse efeito, realizou-se uma análise de Spatial Autocorrelation com as populações totais e separadas por sexo. De seguida, mais uma análise deste grupo foi realizada (Structure Tessellation) para verificar a estrutura genética espacial ao longo da área protegida, calculando-se o número máximo de grupos genéticos de cada população. Posteriormente, analisou-se o padrão de dispersão das duas populações, com estimativas do Mean Corrected Assignment Index (mAI_c) a nível do parque, dos seus diferentes blocos e transectos de amostragem. Finalmente, foi simulada a história demográfica de ambas as populações, estimando o tamanho efetivo da população ancestral (N_1) comparativamente ao mesmo no presente (N_0), e inferindo há quanto tempo essa alteração demográfica terá acontecido (T).

De acordo com os resultados, ambos os colobíneos apresentam populações geneticamente diversas, sendo a população do cólobo vermelho-de-bay mais diversa que a do cólobo preto-e-branco. O último também apresentou um coeficiente de endogamia elevado, ao contrário do cólobo vermelho-de-bay. Relativamente à estrutura genética das populações, a subestrutura existente na população do cólobo preto-e-branco foi confirmada como um enviesamento originado por um conjunto de indivíduos aparentados, enquanto a população de cólobo vermelho-de-bay não aparentou qualquer padrão de estrutura genética. A PCA confirmou esta ausência de estrutura para ambas as espécies e o padrão de IBD revelou-se significativo nos cólobos pretos-e-brancos, mas não nos cólobos vermelhos-de-bay. Quando se consideraram as localizações das amostras, a análise de Spatial Autocorrelation revelou proximidade genética mais significativa que esperado nas distâncias mais curtas para todos os testes de ambas as populações. Além disso, os indivíduos de cólobo preto-e-branco revelaram-se significativamente geneticamente distantes dos que se encontram mais distantes geograficamente, tanto na população total como nos machos e fêmeas separados. No caso dos cólobos vermelhos-de-bay, a população apresentou um padrão complexo de movimentação espacial, repetindo um padrão de proximidade ao nível do grupo social e aos 17 km de distância, seguida por dissimilaridade aos três e 27 km de distância. As fêmeas repetiam o padrão ao nível dos 3 km e os machos demonstravam o padrão de dissimilaridade referente às distâncias maiores. Na análise de Structure Tessellation, verificou-se mais subestrutura nos cólobos pretos-e-brancos, considerando-se três conjuntos genéticos: um referente ao grupo de parentes e outros dois de origem possivelmente antropogénica e histórica. Contrariamente, os cólobos vermelhos-de-bay não apresentaram qualquer subestrutura nesta análise, confirmando assim uma população panmítica nesta espécie. A análise do padrão de dispersão não revelou resultados significativos, apesar das tendências de cada população corresponderem geralmente aos padrões espectáveis para cada espécie. A simulação da história demográfica revelou que as populações dos dois cólobos terão sido grandes no passado, tendo sofrido possivelmente um decréscimo demográfico de uma ordem de grandeza entre a fase Subboreal e Subatlântica do Holoceno no caso dos cólobos pretos-e-brancos, e entre as fases Atlântico e Subboreal do Holoceno no caso dos cólobos vermelhos, ambos provavelmente originados por alterações bioclimáticas.

Os resultados aqui referidos encontram-se em conformidade com o que se conhece de outras populações da África Ocidental, às quais realizou-se uma comparação. O Parque Nacional de Cantanhez (PNC) (Guiné-Bissau) contém elevados níveis de presença humana e florestas severamente fragmentadas, enquanto o Parque Nacional de Taï (PNT) (Costa do Marfim) e o GRNP apresentam habitats florestais contínuos, tendo pouca ou nenhuma presença humana permanente respetivamente. Os dados de diversidade genética apresentados aqui são contrastantes aos do PNC, estando mais próximas das populações mais diversas do PNT. Relativamente à subestrutura, nenhuma das populações das três áreas protegidas revelou uma forte estrutura populacional – um resultado espectável para as florestas contínuas do GRNP e TNP, mas surpreendente no

caso do PNC. Já os resultados de história demográfica não demonstraram alterações demográficas para as populações do TNP, mas mostrou *bottlenecks* demográficos recentes no PNC e antigos no caso do GRNP.
A comparação dos padrões genéticos de cólobos habitantes dos três locais aqui discutidos apontam indiretamente para as consequências das atividades humanas nas áreas protegidas, demonstrando o impacto que estas podem ter em espécies ameaçadas e nos respetivos habitats. Sendo que os primatas arborícolas são altamente dependentes do habitat florestal, o estado genético das suas populações no GRNP reflete a integridade e qualidade da área protegida. Deste modo, reforça-se aqui a importância da manutenção de habitat florestal contínuo para a proteção destes primatas. Os resultados deste estudo poderão ser usados no planeamento da conservação da paisagem internacional de Gola, envolvendo todos os atores interessados e afetados pelo último.

Palavras-chave: primatas arborícolas / macacos cólobos / genética da conservação / preservação florestal / impactos antropogénicos

List of Abbreviations

A_r – Allelic richness
bp – Base pairs
BP – Before Present
BWC – Black-and-White Colobus
BYM – Besag, York and Mollièe (Model)
CAR – Conditional Auto-Regressive (model)
CNP – Cantanhez National Park
CSSL – Conservation Society of Sierra Leone
CR – Critically endangered (IUCN Red List Categories of species classification)
DD – Data Deficient (IUCN Red List Categories of species classification)
DIC – Deviance Information Criterion
DNA – Deoxyribonucleic Acid
EN – Endangered (IUCN Red List Categories of species classification)
FAO – Food and Agriculture Organization (of the United Nations)
FCA – Factorial Correspondence Analysis
FCT – Fundacão para a Ciência e Tecnologia
FEC – Forest Edge Communities
F_{is} – Inbreeding coefficient
F_{st} – Fixation index
GFNP – Gola Forest National Park
GFP – Gola Forest Program
GPS – Global Positioning System
GRNP – Gola Rainforest National Park
H_e – Expected Heterozygosity
H_o – Observed Heterozygosity
HPD – Highest Posterior Density Interval
HWE – Hardy-Weinberg Equilibrium
IBD – Isolation by Distance
IGC – Instituto Gulbenkian de Ciência
IUCN – International Union for Conservation of Nature
K – Optimal number of genetic clusters (in a cluster analysis)
Kmax – Maximal number of genetic clusters (in a cluster analysis)
LC – Least Concern (IUCN Red List Categories of species classification)
LD – Linkage Disequilibrium
Ma – Million years ago
MAFF – Ministry of Agriculture, Forestry and Food Security
mAI_c – Mean Corrected Assignment Indices
MCMC – Markov Chain Monte Carlo
MPSRF – Multivariate Potential Scale Reduction Factor
mtDNA – Mitochondrial DNA
N_a – Number of different alleles
N_0 – Current effective population size
N_1 – Past effective population size
N_e – Number of effective alleles
NP – National Park

NT – Near Threatened (IUCN Red List Categories of species classification)
NTFPs – Non-Timber Forest Products
PCA – Principal Component Analysis
PCR – Polymerase Chain Reaction
PVA – Population Viability Analysis
QI – Quality Index
r – Autocorrelation coefficient
RC – Red Colobus
REDD – Reducing Emissions from Deforestation and Forest Degradation
RSPB – Royal Society for the Protection of Birds
SE – Standard Error
T – Time (years)
TNP – Taï National Park
UV – Ultra-violet
VU – Vulnerable (IUCN Red List Categories of species classification)

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1 Introduction

1.1 Anthropogenic impact on biodiversity

It is evident that life on Earth has been facing an unprecedented crisis in the recent (still unofficial) Anthropocene epoch, characterized by a clear global dominance of human primates over the environment (Dyke & Lamb, 2020). The unnecessary overconsumption and unsustainable reaping of ecosystems' resources by this planetary force has been growing in the context of an expanding industrial agriculture, industrialization, and urbanization. Behind it is the intensified consumerism demanded by unchecked economic growth, which indirectly invades, fragments, and razes natural environments. These actions alter complex biogeochemical cycles and ecosystems, disrupting their normal and natural functions (Rockström et al., 2013). Coupled with humans' overharvesting of organisms through logging, hunting, fishing, and illegal trading, there have been substantial modifications to the ecosphere that sustains us (Norris et al., 2010). These changes have touched the world universally, with the most biodiverse places and low-income communities and nations being disproportionately affected (Levy & Patz, 2015). Consequently, research and restoration of the "wild" species and ecosystems should consider the human dimensions that have brought them upon the biosphere (Vitousek et al., 1997).

Species extinction is the ultimate and irreversible consequence of ecosystems' disruption. Although it is a natural process that precedes humans, the current trends in biodiversity loss and extinctions have accelerated dramatically, with human activities as the main cause. It has been estimated that the current rate of species extinction is 1000 times greater than the background rate of extinction of 0.1 extinctions per million species-years (Pimm et al., 2014). Changes in land use through agriculture and overharvesting of wild species are thought to be the main culprits of biodiversity loss, although it is important to take into account understudied species and interactions between the different threats (e.g., climate change, which may have more impact than previously thought) (Maxwell et al., 2016; Norris et al., 2010). Intensification of agriculture is a short term "solution" to spare forests from agricultural expansion (especially in tropical regions), as it leads to water pollution, further forest clearing due to soil degradation, loss of biodiversity and increasing conflicts with wildlife (Bersacola et al., 2021a; Estrada & Garber, 2022; FAO, 2022; Hockings et al., 2020; IUCN, 2015). Besides the human-induced changes in land use, the introduction of exotic species, pollution, climate change and consequent modifications imposed on the environment further exacerbate the problem (Maxwell et al., 2016). Logging is one of the major landscape-changing human activities, either presenting itself as a consequence of other forms of land use modifications (e.g., agriculture, urbanization, mining) or as fuelwood extraction (both domestic and industrial) (Mallon et al., 2015). Usually, it begins with an encroachment on and isolation of the forested areas and consequent degradation of their edges (also known as edge effect) – which changes their microclimate, soil and vegetation composition, and overall biodiversity (Gascon et al., 2000). This carving up of the woods exposes them to natural destructive phenomena and to further human exploitation of natural resources and land conversion, creating a feedback loop of degradation of forest habitat (Mallon et al., 2015; Volpato et al., 2020). This process is known as fragmentation, where the degradation of habitats eventually leads to the creation of mosaics of man-made landscapes and forest islands (Gascon et al., 2000; Haddad et al., 2015).

The unsustainable harvesting of forest products is destroying livelihoods, carbon sinks, human health and even people's cultural fabric, while simultaneously diminishing the biodiversity of organisms on this planet and disrupting biogeochemical cycles (Estrada & Garber, 2022; FAO, 2022; Volpato et al., 2020). This encroachment on rich and endangered ecosystems led to an urgence in the identification and investigation of priority zones to insist on their conservation: hotspots (Mittermeier et al., 2004; Myers, 2003). These areas are characterized by high demographic density and growth (Bradshaw & Brook,

2014; Veech, 2003), accompanied by exceptionally high levels of biodiversity that show dramatic declines (Brooks et al., 2002; Cincotta et al., 2000). More specifically, to be considered a conservation hotspot, the area must contain at least 0.5% of the world's plant species as endemic and have lost 70% of its primary forest (Myers et al., 2000). That is a recurrent trend in the tropics, where deforestation rates are the highest and are still rising (Hansen et al., 2013), while the creation of protected areas is not a guarantee of protection of biodiversity (Clerici et al., 2007; Laurance et al., 2012). Currently, there are 36 delimited hotspots and most of them are concentrated in the tropics, where demographic pressure, poverty, corruption, civil conflicts, and food shortages aggravate the maintenance of natural ecosystems and hinder conservation efforts (Habel et al., 2019). Many of these biodiversity hotspots are also home to several primate species. However, in various biodiversity-rich nations, conservation can hardly be a high priority considering their levels of poverty, political instability, human population growth and foreign debt (Chapman et al., 2006; Isabirye-Basuta & Lwanga, 2008).

1.2 Guinean Forests of West Africa Hotspot

Habitat destruction and biodiversity loss are more obvious and significant in the case of West Africa, with estimates suggesting that by 2010, 80% of the original forests of the region have been transformed into agriculture-forest mosaic landscape with projections of continued increase (Norris et al., 2010). This is one of the factors that led to the delimitation of the Guinean Forests of West Africa as a Biodiversity Hotspot (Figure 1.1). (Myers et al., 2000). Covering 626,398 km², the region is divided into the "Upper Guinean Forests" that stretch from Guinea, Sierra Leone, Liberia, Côte d'Ivoire, Ghana, to Togo and Benin, separated by the Dahomey Gap (Salzmann & Hoelzmann, 2005) from the "Lower Guinean Forests", which extend through Nigeria, Cameroon, islands of Equatorial Guinea, and São Tomé and Principe (Habel et al., 2019; IUCN, 2015). Although most of the vegetation cover has been lost, it is still habitat to high numbers of endemic species, possessing enormous amounts of biodiversity and species richness. It is home to at least 416 mammal species, of which 65 are endemic – almost one fourth of all mammals native to continental Africa (IUCN, 2015). The Guinean Forests Hotspot is also among the world's priority sites for the conservation of non-human primates (hereafter primates), since 92% of the species found here are endemic, of which five are Critically Endangered and 21 are Endangered (Mittermeier et al., 2004). In the last publication of the top 25 most threatened primates in the world, four species of primates (*Pan troglodytes verus*, *Cercopithecus roloway*, *Colobus vellerosus* and *Piliocolobus epieni*) out of five in continental Africa have their range overlapping with this hotspot's area (Schwitzer et al., 2019). The hotspot's forests contain 9,000 species of vascular plants (20% endemic) also supporting 917 bird species (5% endemic), 1281 freshwater fish species (35% endemic), 269 amphibian species (>30% endemic) and 107 reptile species (~25% endemic). Some of these include notable species such as the Jentink's (*Cephalophus jentinki*) and zebra (*Cephalophus zebra*), duikers, the Diana (*Cercopithecus diana*) and Preuss's (*Cercopithecus preussi*) monkeys, the pigmy hippopotamus (*Choeropsis liberiensis*), the chimpanzee (*Pan troglodytes*), the western gorilla (*Gorilla gorilla*), the Taï toad (*Amietophrynus taiensis*) and the cherry mahogany (*Tieghemella heckelii*). These prominent species, along with many complex ecological features, render this hotspot globally outstanding in terms of biodiversity (IUCN, 2015).

Figure 1.1 Map of the Guinean Forests of West Africa Biodiversity Hotspot, illustrating its division between the "Upper Guinean Forests" and "Lower Guinean Forests" by the Dahomey Gap. Original map from the Global Forest Watch (www.globalforestwatch.org), adapted by author.

There is evidence that more than 85% of native vegetation cover in this hotspot has been lost (Mittermeier et al., 2004), with recent estimates pointing to only 10.6% of remaining cover (Habel et al., 2019). As in many tropical areas, the need to provide for a growing human population in the rural and urban context has been pressuring for agricultural expansion (slash-and-burn, industrial, plantations), wood extraction, infrastructure extension, industrial development, logging, fishing, mining and bushmeat hunting (Norris et al., 2010). Agriculture is the major economic sector in all the hotspots' countries, providing sustenance for the growing human population and for commercial export, contributing significantly to land-use change and deforestation (IUCN, 2015). This region is thought to have approximately 84,700,000 people (137 persons/km²), with around 5,000,000 of them living at a short distance (less than 10 km) from a protected area (127 persons/km²) (Mittermeier et al., 2004). Therefore, the local communities are highly dependent on the ecosystem services of the forests, but the need for extraction of resources has been the main origin of fragmentation of said forests, which is directly connected to biodiversity loss (Norris et al., 2010). The consequences of all these activities include pollution and destruction of habitat for many species (including endemic ones) and worsening of climate change effects – which wreak havoc on biodiversity, especially when combined with political instability, conflicts, and extreme levels of poverty (Mallon et al., 2015; Veech, 2003). These effects critically impact the human populations too, as their survival has always depended greatly on the ecosystem services of the forests surrounding them (FAO, 2022). Forest resources are vital for people, including for subsistence, income generation, and medicine in the hotspot countries (IUCN, 2012). Concurrently, they have been suffering exponentially with the deterioration of the natural resources they depend on, while the consequences of climate change have been and are expected to continue affecting them disproportionately (IPCC, 2022).

1.3 Threats to primates

Currently, over 65% of the world's primate species are under threat according to the IUCN Red List classifications of Vulnerable (VU), Endangered (EN), or Critically Endangered (CR), with 93% presenting declining populations (Estrada & Garber, 2022; Fernández et al., 2022). The main threats to their populations include deforestation, unsustainable hunting for bushmeat and the pet trade, expansion of transportation infrastructure, mining, dam building, and fossil fuel extraction (Estrada et al., 2020).

There are also threats that are thought to be more dangerous now and to become more prominent in the future, including climate change (Meyer & Pie, 2022; Zhang et al., 2019) and the emergence of new diseases (Fernández et al., 2022). The primate species listed as the world's top 25 most endangered have habitat destruction/degradation (particularly tropical forests) and hunting (both bushmeat and illegal wildlife trade) as main threats (either both threats or one of them) (Schwitzer et al., 2019). The effects of anthropogenic disturbance on their populations are directly connected to the major threats to their survival (Cavada et al., 2019), with some authors arguing for the inclusion of a separate index of threat – human density – to the criteria used to indicate a species' threat status in the IUCN Red List (Harcourt & Parks, 2003). Additionally, the generally higher incidence of primate presence in areas of lower elevation situates them even closer to human settlements (Cavada et al., 2019). Proximity between human and non-human primate communities may lead to conflict and competition for resources between humans and primates (Bersacola et al., 2021; Hockings et al., 2020; Parathian et al., 2018), and further expose the latter to habitat reduction/fragmentation, isolation, hunting and even zoonoses (Estrada et al., 2017; Hockings et al., 2015; Hockings & Sousa, 2013). Moreover, the most frequent system of legal protection of natural habitat does not shelter many populations and species, since 94 primate species worldwide are thought to have less than 10% of their distribution in officially protected areas (Estrada & Garber, 2022). Even within protected areas, threats to wildlife such as hunting, artisanal mining, and capture of primates for illegal pet trade continue to exist (Fernández et al., 2022). Furthermore, their conservation is also difficult due to the fact the nations where most species are extant, are also economically and/or politically unstable, while most of the extraction of their natural resources serves to feed a global market demand for export to rich countries (Estrada et al., 2020; Estrada & Garber, 2022; Isabirye-Basuta & Lwanga, 2008). These aspects, along with crushing foreign debts and the highest human population growth rates, hinder conservation efforts in many of these nations. Finally, the threats are sometimes interrelated and happening in synergy, increasing the overall negative impact on both primates and their habitats (Estrada et al., 2017).
Pressure from human activities can vary throughout different regions; this is true for drivers of deforestation, which can be mainly commodity-driven, long-term deforestation, such as in the Americas (56%) and Asia (78%), while in sub-Saharan Africa, small-scale agriculture and consequent short-term forest clearing is the main driver of deforestation (92%) (Curtis et al., 2018). Primate vulnerability to deforestation depends on both their specific biology and the origin and level of deforestation. Body size, diet type, and the degree of ecological flexibility to habitat disturbance are traits that can make a difference in the risk of extirpation (Isaac & Cowlishaw, 2004). Most primates are considered sensitive to habitat disturbances due to their rarity, dispersal modes and needs, long and complex life histories, resource and range requirements, trophic level, and high degree of specialization (Chapman et al., 2000; Estrada et al., 2017; Harcourt et al., 2002; Hockings et al., 2015; Kalbitzer & Chapman, 2018; Marshall & Wich, 2016; Oates, 1996; Pearson et al., 2014; Struhsaker & Leland, 1979). However, several primate species also present some degree of behavioral flexibility, which may facilitate responses to changes in the habitat (Chapman et al., 2002; Estrada et al., 2017; Isaac & Cowlishaw, 2004; Kalbitzer & Chapman, 2018; Nowak & Lee, 2013). On the other hand, many of these adaptations are only short-term responses, and some behaviors may endanger the animals through higher levels of exposure to human populations (Hockings et al., 2015).
Deforestation has been studied in several different perspectives and distinguished by origin, extension, and duration, but it is not always clear why some species of primate persist while others disappear in forest fragments. Some reasons have been suggested, such as the resilience of species with high ecological flexibility in areas where selective logging occurs, the success in coping with impacts of shifting cultivation by terrestrial frugivorous species and the vulnerability of large primates to hunting (Isaac & Cowlishaw, 2004). Even within the same species and the same locations, different responses to fragmentation are recorded (Isaac & Cowlishaw, 2004; Isabirye-Basuta & Lwanga, 2008). For example, forest fragmentation creates edge effects that lower the quality of the forest habitat and further expose

animals to other threats such as hunting, resource extraction, mining, industrial and urban expansion, while also decreasing the resilience of the forest to climate change (Galán-Acedo et al., 2019). With time, the same urban and industrial expansion facilitates further deforestation, limiting the movement of animals between the isolated forest areas, as well as their access to resources and other populations, exposing them more frequently to human activities (Ascensão et al., 2022).

This complexity of land use mosaics and primate responses to threats adds difficulties to their study in changing habitats (which are the norm), as the reasons for their behavior are not always clear. For example, populations of blue monkeys (*Cercopithecus mitis*) and red colobus (*Procolobus pennantii*) are declining without any signs of logging in Ngogo and Kibale National Park, Uganda (Mitani et al., 2000). In the case of the former, this result is expected because of a previous study that revealed that blue monkeys – being generalists – have a hard time competing with specialists of old growth forests, showing population decline with time (Struhsaker & Leland, 1979). This suggests that as the forests grow old, these monkeys start disappearing until their populations are no longer viable. The largest population of chimpanzees also lives in this forest and appears to be taking advantage of the fact that it was previously inhabited by humans, who planted a significant amount of food trees (Isabirye-Basuta & Lwanga, 2008). At the same time, these animals have been shown to hunt red colobus there, this being the greatest threat to the colobines' survival in this context (Teelen, 2008). With these examples, we can observe how, without knowledge of the forest's history, what are fluctuations in primate communities responding to natural phenomena could have been interpreted as a sign of logging; unfortunately, there are not many multi-species studies in one forest (Isabirye-Basuta & Lwanga, 2008). Furthermore, studies on primates are usually focused on some species and sites, while many still lack scientific data, let alone detailed studies spanning several decades such as the example of primates in Kibale National Park (Estrada et al., 2017).

In another study focusing on logged (1980-1997) and unlogged (1970-1997) areas of Kibale National Park (Uganda) researchers examined the effect of habitat changes on several primates. They concluded that the eastern black-and-white colobus (*Colobus guereza*) groups migrated to parts of previously heavily logged forests, which initially appeared to reduce the relative abundance of the studied populations. However, what happened was an increase in population density and a shift of the populations' home range into the heavily logged area (Chapman et al., 2000). The authors' findings in that context also suggest a continuous decline in blue monkey (*Cercopithecus mitis*) and red-tailed monkey (*Cercopithecus ascanius*) populations even 28 years after logging activities stopped, pointing out that the "unexpected decline" in population numbers in a recovering forest may be due to a plethora of reasons. These can vary from the tree species that compose the forest and the origin of their modification along the years, the biology, behavior, habitat and food preferences of the primates, the impact of large herbivores such as elephants and even the detectability of the animals by the observers upon thickening of the recovering forest. Such observations are useful to highlight the consequences of deforestation and simultaneously remind that there may be other synchronous threats that have detrimental effects on the primate populations (Chapman et al., 1997).

Considering primates' difficult situation in the Anthropocene, it is important to remember that they are relevant elements in some human cultures (Parathian et al., 2018), contributing also to the understanding of our shared evolutionary history, psychology, and neurology (Estrada et al., 2017; Marshall & Wich, 2016). They also play a significant role in the functionality and quality of the forest ecosystem – partaking in seed dispersal and predation, pollination, tree regeneration, predator-prey relationships – maybe even in buffering forests against the detrimental effects of climate change (Marshall & Wich, 2016). They are, at the same time, one of the most speciose in the mammalian context (Estrada et al., 2017) and concomitantly the most well-studied group of animals in the tropical areas (Marshall & Wich, 2016); yet there is still a lot we do not know about our closest relatives.

1.4 The *Colobinae*

The *Cercophithecidae* family of Old-World monkeys is divided into the *Colobinae*, commonly known as colobines or leaf-eating monkeys, and the *Cercopithecinae*, also known as cercopithecines or cheekpouched monkeys (Delson, 1975). The former, *Colobinae*, are known for their adaptations to a folivorous diet – such as a complex, multi-chambered stomach – although what better distinguishes them is their absence of a thumb (Groves, 2007). During the Miocene (about 12 Ma), they radiated into the African and the Asian clades of colobines, but only in the Pleistocene appeared the first colobines of similar morphology to the currently extant species (Ting, 2008). Notwithstanding the different classifications, the African colobines are divided into three genera: the *Procolobus*, also known as olive colobus, the *Colobus*, or black-and-white colobus, and the *Piliocolobus*, commonly known as red colobus (Delson, 1975; Groves, 2007). A study using mitochondrial DNA indicates that the separation of the black-and-white colobus from the other colobus monkeys occurred 7.5 Ma, and the remaining taxa (red and olive colobus) indicated a divergence at 6.4 Ma (Ting, 2008). This diversity and divergence could have originated in past glacial periods, where the monkeys had to seek refuge from the adverse climatic conditions. Once these periods ended and the climatic conditions became more favorable, the monkeys would radiate and adapt to these different forest habitats (Minhós, 2012).

According to recent literature, colobines are globally affected by 11 out of 12 major threats as identified by the IUCN Red List, which makes them one of the most threatened (by number of threats) of all primate taxonomic groups (Fernández et al., 2022). The patterns of colobine distribution and abundance across the landscape are influenced by the availability of food and the protein-fiber ratios of the food items (Chapman et al., 2002; Chapman et al., 2004). Due to their arboreal lifestyle and folivorous diets, African colobines are particularly affected by drastic habitat degradation (Minhós et al., 2016), as illustrated by the fact that the first primate declared possibly extinct in the 20th century was Miss Waldron's red colobus (*Piliocolobus waldronae*) (Oates et al., 2020). Still, some red colobus species have been recorded showing behavioral and dietary adaptations in response to the changing habitat (Galat-Luong & Galat, 2005; Nowak, 2008), while black-and-white colobus show some persistence and even success in surviving in disturbed habitats (Chapman et al., 2000; Klop, 2008; Minhós et al., 2016).

The taxa of this study are the black-and-white colobus or King colobus (*Colobus polykomos*), belonging to the genus *Colobus*, and the Bay colobus (*Piliocolobus badius badius*), which belongs to the genus *Piliocolobus* (Groves, 2007). Both are classified as endangered by the IUCN and present a decreasing trend in their populations' number (Gonedelé Bi et al., 2019; McGraw et al., 2020). Due to their high dependence on the forest habitat, these arboreal species are good indicators of the ecosystem's overall status (Hillers & Tatum-Hume, 2013). The two species of colobus share some degree of similarity regarding their diet and ecology, leading to instances of cohabitation in the same geographic range, or sympatry (Davies et al., 1999; Minhós, Nixon, et al., 2013a; Minhós et al., 2016; Sterck et al., 2002). However, there are also socioecological differences regarding their social group size (larger in red colobus than in black-and-white's), dispersal patterns (both sexes disperse in black-and-white colobus, primarily females in red colobus) and dietary flexibility (greater in black-and-white than in red colobus) (Korstjens & Dunbar, 2007; McGraw et al., 2015). Their biogeographical similarity and simultaneous contrasting socio-ecological characteristics make them useful models to study the determinants of dispersal behavior (Minhós et al., 2013a).

1.4.1 The *Colobus* Genus and *Colobus polykomos* (Zimmerman, 1780)

The genus *Colobus* had five recognized species, distributed throughout equatorial Africa (Figure 1.2): the least-concern (LC) species *Colobus guereza*, the vulnerable (VU) *Colobus angolensis* and *Colobus satanas*, the endangered (EN) *Colobus polykomos*, and the critically endangered (CR) *Colobus vellerosus* (Groves, 2007; IUCN, 2022). Additionally, one subspecies known as the Mount Kilimanjaro guereza (*C. guereza caudatus*) has gained the species rank under the *Colobus caudatus* designation

(Butynsky & de Jong, 2018) and was more recently classified as Vulnerable in the IUCN Red List (de Jong et al., 2020). Their fur coloration is one of the characteristic traits of these colobines – black pelage (only color in the case of *C. satanas*) with diverse combinations of white and grey on the animals' tail, thighs, shoulders, and head (Groves, 2007).

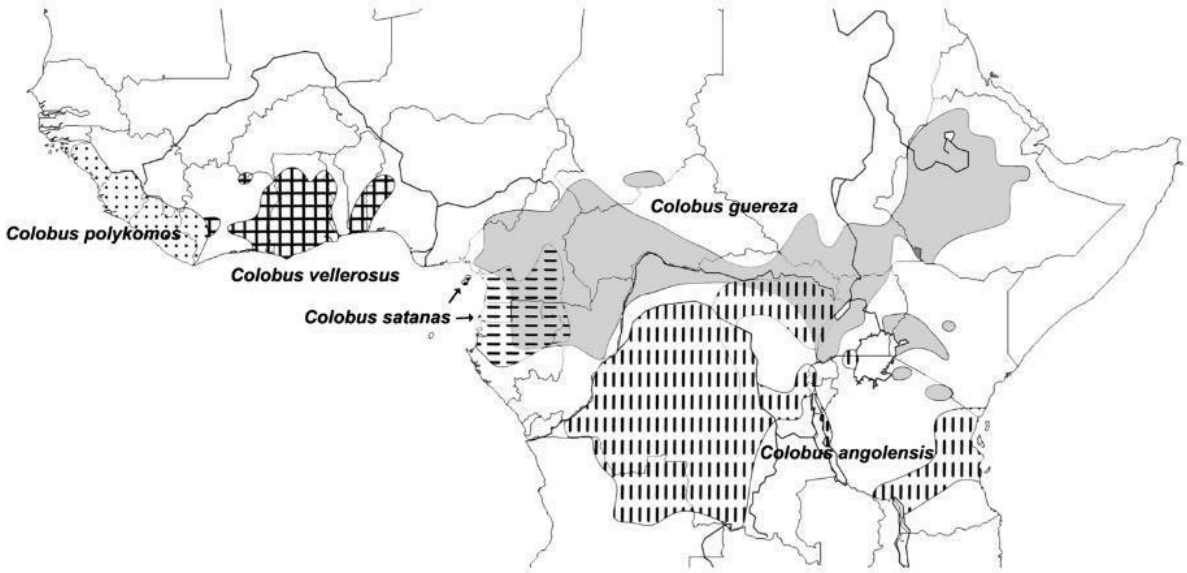


Figure 1.2 Distribution map of the black-and-white colobus species (*Colobus* genus), from Ting, 2008.

The animals of this genus usually form multi-male multi-female social groups composed by no more than 20 individuals – except for the mostly polygynous *C. guereza* and *C. polykomos*, that can form unimale groups and where both sexes can disperse (Minhós et al., 2013a; Sterck et al., 2002). These arboreal and folivorous primates inhabit various types of forest, with *C. guereza* and *C. polykomos* exhibiting some toleration to habitat disturbance (Chapman et al., 2000; de Jong et al., 2019; Minhós et al., 2016). Notwithstanding their adaptations to habitat change, all black-and-white colobus species show decreasing populations. Habitat loss, degradation, and fragmentation (due to logging, expansion of agriculture and infrastructure for various industries) is one of the primary threats to their survival. Hunting is another relevant threat to these colobines, especially for *C. satanas*, *C. polykomos* and *C. vellerosus*, but the overall growing pressure from human populations and their activities is always a threat to these primates (de Jong et al., 2019; de Jong et al., 2016, 2020; Gonedelé Bi et al., 2019; Goodwin et al., 2020; Maisels & Cronin, 2019).

Colobus polykomos (Figure 1.3) can be found in rainforests and gallery forests of West Africa, more specifically Guinea-Bissau, Guinea, Ivory Coast, Liberia, and Sierra Leone (Gonedelé Bi et al., 2019). They feed on seeds, young leaves and occasional flowers when resources are plentiful, and incorporate old leaves into their diet during periods of food scarcity (Davies et al., 1999; Korstjens & Dunbar, 2007). Although this makes them highly dependent on the forest habitat, they have shown some degree of resilience to changes – accepting to stay in smaller patches of forest, modifying their group size and dispersion, as well as their diets (Gonedelé Bi et al., 2019; Minhós et al., 2016).

Figure 1.3 *Colobus polykomos*, photograph by Liz Mulligan (2011).

However, when the animals have a choice between staying in a more pristine versus a more degraded habitat, they tend to choose the former (Klop et al., 2008). They usually form uni-male groups of 10 to 16 individuals, where males are usually the dispersing sex and females are more philopatric (Minhós et al., 2013a) – although both sexes have been reported to disperse (Sterck et al., 2002). This occasional female dispersal behavior has been rationalized to be related to food-seeking (Korstjens et al., 2005), and may indicate adaptations to environmental change (Minhós et al., 2013a). Their populations are mainly threatened by hunting throughout their range, where the destruction, degradation and fragmentation of forest habitat has also been pressuring them to disappear (Gonedelé Bi et al., 2019; Oates, 1996).

1.4.2 The *Piliocolobus* genus and *Piliocolobus badius badius* (Kerr, 1792)

The taxonomic classification of red colobus species is not as simple as the black-and-white's, since the complex patterns of their pelage, vocalizations and cranial morphologies have obstructed the study of their evolutionary connections (Cardini & Elton, 2009; Grubb et al., 2003; Oates & Ting, 2015). Some authors have chosen to distinguish the red colobus monkeys and the olive colobus monkeys as separate genera – *Piliocolobus* and *Procolobus* respectively (Groves, 2007). Others consider them to belong to one genus – *Procolobus* – with to subgenera (*Piliocolobus* for the red colobus and *Procolobus* for the olive colobus), a taxonomy followed by the IUCN (Grubb et al., 2003). A molecular study by Ting (2008) indicated the divergences between the three genera/taxa of colobines also exposed the existence of three major clades within red colobus, that split 3 Ma. Here, the mtDNA sequences of all commonly acknowledged subspecies revealed a clade in West Africa (*P. b. badius* and *P. b. temminckii*), another one in the Western Equatorial Region and the Congo Basin (*P. b. pennantii*, *P. b. preussi* and *P. b. tholloni*) and a final one in Central Africa (*P. b. rufomitratus*, *P. b. tephrosceles*, *P. b. tholloni* and a sister taxon including *P. b. kirkii* and *P. b. gordonorum*). The author placed all the red colobus as subspecies of *Procolobus* (*Piliocolobus*) *badius.* These previously considered subspecies of *Piliocolobus badius* are presently accepted as species, which are distributed throughout the African West to East (Figure 1.4). This great diversity in the red colobines might have its origins in the isolation of populations in refugia, where evolutionary pressure forced divergence in their morphologies (Cardini & Elton, 2009). Although species have their particularities, they have in common a folivorous diet determined by protein-fiber ratios, which is composed mainly by young leaves and fruits when possible and mature leaves when the former are scarce (Korstjens & Dunbar, 2007; McGraw et al., 2015). Additionally, all species present pelage patterns with diverse amounts of red, black, white, gray, and brown colors, with some variations detected also at the population level (Struhsaker, 2010).

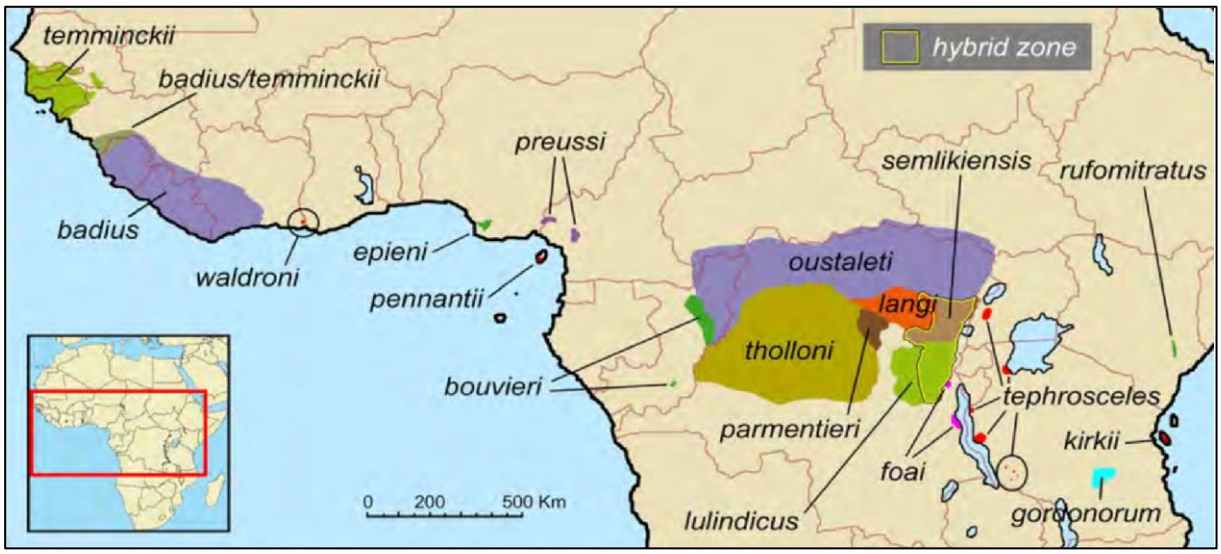


Figure 1.4 Distribution map of the red colobus species (Piliocolobus genus), by Stephen D. Nash, in Linder et al., 2021.

They gather in large social groups, typically between 25 and 40 individuals – but may exceed 80 animals (Minhós et al., 2013a). Their large social groups and interspecific relationships with other primates (Galat-Luong & Galat, 2005; Noë & Bshary, 1997) may be explained by their high predation pressure from chimpanzees, with which they have a predator-prey relationship in many areas of distribution (Boesch, 1994).

The Western Red colobus or *Piliocolobus badius* (EN) (McGraw et al., 2020a) has two subspecies: the Temminck's Red colobus (*Piliocolobus badius temminckii*) (Minhós et al., 2020) and the Bay colobus (*Piliocolobus badius badius*) (Figure 1.5) (McGraw et al., 2020b), the latter being the object of this study and to which I will be further referring to when talking about the red colobus. Along with *C. polykomos*, they represent the westernmost taxa of African colobus monkeys (Minhós et al., 2016). *P. b. badius* is present in southern Guinea, Ivory Coast, Liberia, and Sierra Leone, but its geographical separation from *P. b. temminckii* is yet unclear, since their distributions may overlap in the south of Guinea and north of Sierra Leone (Figure 1.4) (Groves, 2007; Linder et al., 2021). The animals inhabit primary, secondary, and gallery forests, woodland, tree and shrub savannas and were also detected in mangrove formations and even residential gardens (McGraw et al., 2020b).

Figure 1.5 *Piliocolobus badius badius*, photograph by Edgar Thissen (2008).

When they have an option, the monkeys have a clear preference for old growth forests; here they subsist on fruits, seeds, young leaves and flowers in times of abundance and on mature leaves when those preferred foods are scarce (Davies et al., 1999; Lindsell et al., 2011). They gather in multimale and multi-female groups ranging from 2 to 60 individuals, with dispersal usually being mediated by females (Binczik et al., 2019; Minhós, Nixon, et al., 2013b; Struhsaker, 2010). The biggest threat to these monkeys is hunting for subsistence and bushmeat trade, followed by deforestation due to logging, mining, charcoal production and farming, which have been growing concomitantly with the human population (Linder et al., 2021). The civil war in Sierra Leone is thought to have had some impact in the red colobus populations (McGraw et al., 2020b). These animals are extremely sensitive to habitat alterations, that if too great, can pressure the monkeys to disappear (Linder et al., 2021; Minhós et al., 2016).

1.5 Study site

1.5.1 Sierra Leone

The study area is located in the southeast of Sierra Leone – of which 65.4% overlap the western extremity of the Upper Guinean Forests of West Africa ecoregion of the Guinean Forests of West Africa Hotspot. The north of the country is mostly surrounded by Guinea, with the Atlantic Ocean on the south and west side, and Liberia on the southeast (IUCN, 2015). With an annual rainfall ranging from 1900 mm in the northwest to more than 4000 mm on the coast, it is one of the wettest countries of West Africa. The region is thus characterized by a humid tropical climate that, along with a diverse terrain from the flat coast to the central plains and to the high eastern mountains, creates the perfect conditions for the development of a complex matrix of mangroves, rain forests, woodlands, savannas, croplands, and pastures.

Like many West African countries, Sierra Leone has been losing forest cover at swift rates: 30% were lost between 1975 and 2013, with an annual rate averaging 0.8% (CILSS, 2016). Since then, and until 2021, tree cover decreased 29% (University of Maryland & World Resources Institute, 2022a), of which 1.7% were humid primary forest (Hansen et al., 2013; University of Maryland & World Resources Institute, 2022b) – with deforestation through shifting agriculture being the dominant driver of this loss (Curtis et al., 2018; The Sustainability Consortium, World Resources Institute, & University of Maryland, 2022). Notwithstanding the different estimations of forest loss (Wadsworth & Lebbie, 2019), the land cover change is a reality – and has its origins in cropland expansion, 'slash-and-burn' agriculture/fire-fallow cultivation, logging, mining, and cattle grazing activities (CILSS, 2016; Government of Sierra Leone, 2017). The nation has been working towards sustainable development and concomitant conservation of its biodiversity through the establishment of protected areas (Government of Sierra Leone, 2017), which at the international level include a Ramsar site (Sierra Leone River Estuary, 1999) and at the national level, 66 protected areas comprising eight national parks, 46 forest reserves, four marine protected areas, three strict nature reserves, four no-hunting forest zones and one game reserve (UNEP-WCMC, 2022).

The country produces many high-demand minerals, but income inequality is extremely high – having had one of the world's highest poverty rates in 2018, when more than half of its population was living on less than $1.90 per day (World Bank, 2021). It is estimated that 8.6 million people live in Sierra Leone, presenting a population growth of an average of 2.49% per annum (CIA, 2022). The country's inhabitants have long been subsisting through agriculture – especially shifting agriculture, which is characterized by the burning of forest patches that are used for one to two years as cropland and then left fallow for several years before being used again (CILSS, 2016). It is projected that 70% of the rural population is dependent on this type of agriculture for subsistence, which is not surprising considering that about 65% of the country's Gross National Product is accounted for by agriculture alone (Government of Sierra Leone, 2017). The structural problems in the country put it in the worlds' lowest ratings of the Human Capital Index (0.4), which in a scale of zero to one, measures the health and education contributions to a future worker's productivity (World Bank, 2021). It is also in the worlds' lowest ranks (184th out of 189 countries) in the United Nations Development Index (0.419) in 2017, a mean of three basic human development dimensions: a long and healthy life, access to knowledge and a decent standard of living (United Nations Development Programme, 2019). Furthermore, the nation has been impacted by a civil war (1991-2002), an Ebola epidemic (2014-2016) and, more recently, the COVID-19 pandemic (2020-2021), which have hindered Sierra Leone's socio-economic progress (World Bank, 2021). These events created intense socio-political instability, increased poverty and food insecurity, and decreased access to education and health services (World Bank, 2021). Specifically, forest reserves were used for camps by the Revolutionary United Front, which greatly targeted and forcibly displaced rural populations (Lindsell et al., 2011). Currently, the recovery has been further compromised by the increase in food and fuel prices as a consequence of the war in Ukraine and deterioration of public finances (World Bank Group, 2022).

1.5.2 Gola Rainforest National Park

Gola Rainforest National Park is situated in the southeast of Sierra Leone (between latitude 07°18'22" N and 07°51'00" N, and between longitude 11°21'13" W to 10°37'40" W, Figure 1.6), at the border with Liberia. Its area of 710,7 km² subdivides into three forest blocks: Gola North, Gola Central, and Gola South. This study includes Tiwai island situated at the northwest of the latter zone as part of the study area. Gola South and Tiwai island lie within the Pujehun and Kenema districts, Gola Central is in Kenema and Kailahun districts and Gola North is in the Kailahun district.

The forest also falls within the limits of seven chiefdoms: Barri and Makpele chiefdoms in the Pujehun district, Koya, Gaura, Tunkia and Nomo in the Kenema district and the Malema chiefdom in the Kailahun district (Klop al., 2008). The forest blocks serve as catchment areas for the Mahoi, Moro, Mano and Moa rivers, which receive about 3000 mm of rainfall throughout the year, especially between July and August (Barca et al., 2018). The area of lowland moist evergreen forest is important in terms of biodiversity conservation, both nationally – due to it being the largest area of lowland moist evergreen forest in Sierra Leone – and internationally – considering that it is included in the Upper Guinean Rainforests ecoregion (Barca et al., 2018; IUCN, 2015). It was officially established as a forest reserve in 1926 and 1930 – although it was commercially explored for timber, mainly throughout the 1960s and 1980s (see Davies, 1987) and possibly had a past of disturbance or a change in growth-promoting environmental conditions before the commercial logging concessions (Lindsell & Klop, 2013). This exploration occurred mostly in the Gola South block due to its more accessible terrain, as well as the Central block – where, after 23 years, it is still possible to see the consequences of the harvest (Kent et al., 2015; Lindsell & Klop, 2013). Contrastingly, the difficult and high terrain (79% of which is over 250 m of altitude) of the Gola North block has deterred such a degree of forest degradation, with about 19% of the block having been exploited. As for Tiwai island, its deforestation had its origin in farming activities, which stopped in 1987 with the declaration of the island as a Wildlife Sanctuary (Klop et al., 2008).

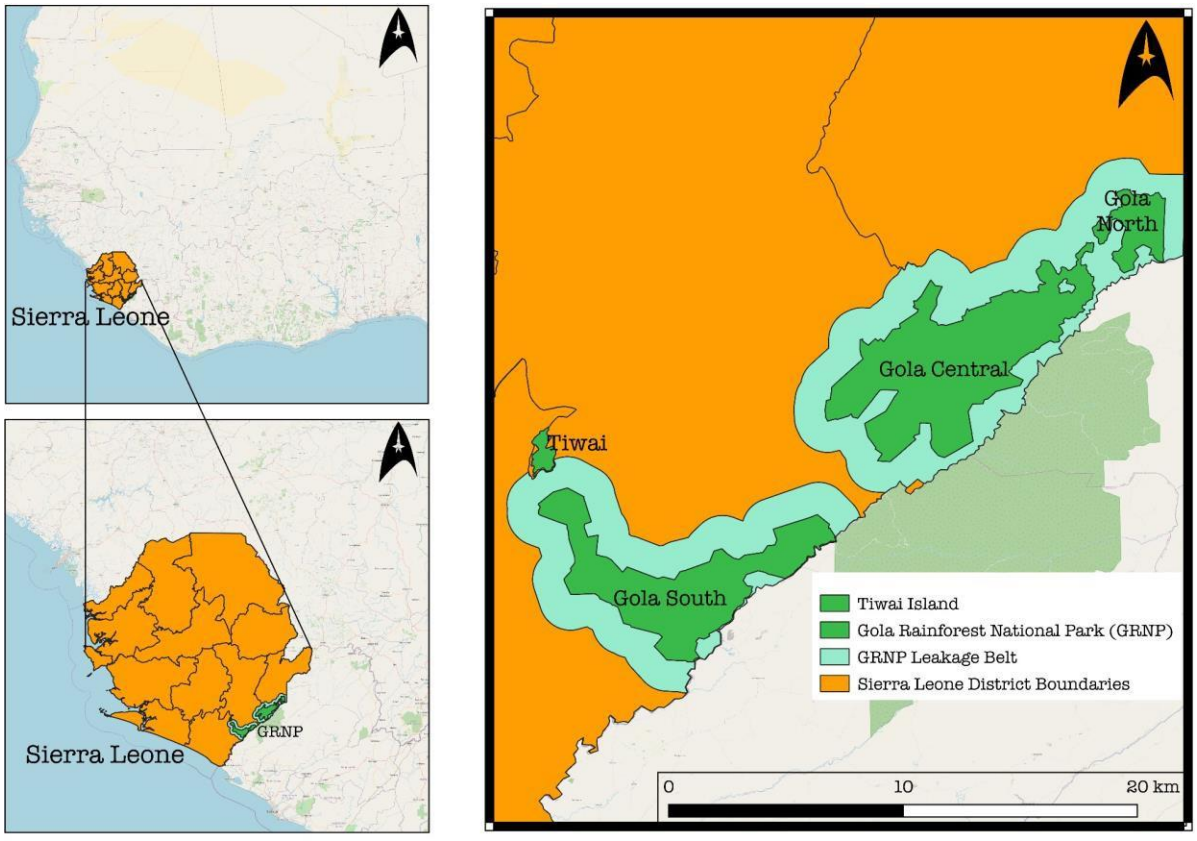


Figure 1.6 Map specifying location of Sierra Leone in West Africa, as well as the Tiwai Island, the Gola Rainforest National Park and its respective leakage belt and blocks within Sierra Leone. Produced in Quantum GIS v. 3.16.10-Hannover.

The protected area of Gola has been managed since 1990 by the Sierra Leonean government's Ministry of Agriculture, Forestry and Food Security (MAFFS), the Royal Society for the Protection of Birds (RSPB-UK) and its local partner, Conservation Society of Sierra Leone (CSSL). The work of this partnership through the Gola Forest Program (GFP) created the opportunity for the forest reserve to obtain National Park status in 2011, in preparation for the establishment of the country's first REDD+ project (Government of Sierra Leone, 2017). From 2017 to 2020, the protected area was granted a contract for the Community Landscape Management project in the context of the West Africa Biodiversity and Climate Change (WA BiCC) program for the Gola Transboundary Forest, which includes not only GRNP, but its biotic continuation into Gola Forest National Park (GFNP) and the proposed Foya Nature Reserve in Liberia. The project has the objective of joining conservation efforts between the two countries for the protection of 3 500 km² of forested landscape and its biodiversity, directly involving the nearby human communities that depend on those forests. Local communities have been trained in agricultural intensification practices, including rainforest-friendly cocoa, lowland rice, groundnut and vegetable production, as well as beekeeping (USAID/WA BiCC et al., 2020). This work is essential to provide tools to create sustainable livelihoods for the forest edge communities (FEC), i.e., communities that border the National Park and affect and are affected by conservation work. In the case of the GRNP, community forests are essential for its inhabitants, who largely identify themselves as Mende (86%), the other ethnicities being mainly Gola (6.3%), Fula, Mandingo, Vai, Kissi, Limba, Gbandi and Temne. There are 122 villages considered FEC, situated within the 4 km-wide leakage belt around the protected area, where an estimated 23 500 people live. Here, 90% of inhabitants depend on agriculture for income, focusing mainly on subsistence rice production and cultivation of cash crops (Bulte et al., 2013; RSPB, 2015). Thus, it is necessary for conservation projects to include the communities, with a view to both bolster the biodiversity of the protected area and alleviate some of the anthropogenic pressure that builds upon it.

These efforts are fundamental to preserve the many species that depend on the forest, which records show has around 899 species of plants, with 232 being tree species (Klop et al., 2008; RSPB, 2015). The vulnerable white-necked Picathartes's (*Picathartes gymnocephalus*) image is the flagship species represented in the protected area's logo, likely because of its vulnerable conservation status and its small distribution range (GRNP is one of the few known permanent habitats for the species). Besides that, birds are very well represented in the GRNP (327 recorded species of birds) – among the highest recorded number of species in the Upper Guinean Forests. Furthermore, 43 amphibian species have been identified, one third of which are listed as endangered (EN) or near threatened (NT) by the IUCN Red List, including the endangered Taï toad (*Amietophrynus taiensis*) (IUCN, 2022; RSPB, 2015). Additionally, 13 reptile species are known to reside here, including the vulnerable (VU) African dwarf crocodile (*Osteolmaemus tetraspis*) and the Forest Hinge tortoise (*Kinixys eros*), which remains data-deficient (DD) (IUCN, 2022; Klop et al., 2008). Concomitantly, the diversity and uniqueness of the fish communities found here are high: 31 species have been found in the rivers, 35% of which are regional endemic to Sierra Leone and Liberia Upper Guinean ecoregion. The same can be said about the butterfly diversity, which may exceed 600 species in the GRNP, out of the 750 present in Sierra Leone, and of the dragonfly and damselfly diversity, representing 80% of all species found in the country. Complete assessments are essential for all invertebrates, as many of them are endemic, endangered, and probably many are yet unknown to science (RSPB, 2015). Mammals, on the other hand, are more frequently studied – with recent surveys indicating about 49 species of large mammals present in the area, such as the Pygmy hippopotamus (*Hexaprotodon liberiensis*), the Jentink's duiker (*Cephalophus jentinki*), the Zebra duiker (*Cephalophus zebra*) and the African Forest Elephant (*Loxodonta cyclotis*). This protected area is also essential for the conservation of many primate species, such as the Demidoff's dwarf galago (*Galagoides demidovii*), the Thomas's dwarf galago (*Galagoides thomasi*), the Potto (*Perodicticus*

potto), the Sooty mangabey (*Cercocebus atys*), the Diana monkey (*Cercopithecus diana*) the Olive colobus (*Procolobus verus*), the Campbell's monkey (*Cercopithecus campbelli*), the Lesser spot-nosed monkey (*Cercopithecus petaurista*), the Green monkey (*Chlorocebus sabaeus*) and the western Chimpanzee (*Pan troglodytes verus*), as well as for the two species presented in this study. According to the literature, the civil war did not seem to affect the mammal fauna of GRNP, which showed little to no sign of reduction in the number of animals (Lindsell et al., 2011). Hopefully, the conservation efforts between Sierra Leone and Liberia will continue, as many (if not all) of the organisms present in the Gola landscape benefit from habitat connectivity and conservation due to their high dependence on the forest ecosystem.

1.6 Conservation genetics

The current biodiversity crisis and species extinction that has been plaguing the biosphere is, as we have seen, a direct consequence of human actions. Conservation biology is one of the solutions sought by humans to reduce these effects, through preservation of wildlife and the ecosystems that support it. In this context, genetic studies on threatened species and populations have been an important toolbox to inform on their biology and ecology, so that conservation planning is done as effectively as possible (Salgado-Lynn et al., 2016). Through genetics, conservation scientists seek to monitor, maintain and/or recover genetic diversity in populations – which minimizes their risk of extinction by lack of adaptive genetic capabilities (Frankham, 2010a, 2010b). This work has become more important than ever, since it permits to obtain DNA from non-invasive samples (hair, food wadges, feathers, urine, feces, etc.) from free-ranging, endangered species (Piel et al., 2022; Taberlet et al., 1999) and obtain the genetic data quickly, effectively, and relatively cheaply (Guichoux et al., 2011). With it, we can answer many questions about a population's size and structure, genetic diversity, dispersal, demographic history, kinship, among many others (Ferreira da Silva et al., 2012; Selkoe & Toonen, 2006). Some of these questions cannot be answered through observational studies alone, especially in long-lived (Orkin et al., 2021), slow reproducing (Goossens et al., 2006), bottlenecked (Groombridge et al., 2000; Robinson et al., 2022) and elusive species (Fernandes et al., 2008), which is the case of some primates (Harris et al., 2009).

Amid the many genetic markers used in these studies – such as microsatellites, allozymes, mitochondrial, SNPs – the former have become a common choice. Microsatellites (also known as short tandem repeats (STR) and simple sequence repeats (SSR)), are short 70 to 200 bp sequences which repeat in tandem (usually between 2 and 6 bp repeated a few dozen times), often located in non-coding regions of the genome. Primers can be designed to bind to the flanking regions of microsatellites and amplify them through a polymerase chain reaction (PCR), allowing geneticists to successfully genotype them. Found abundantly in the nuclear DNA, they are generally considered ideal Mendelian markers, since they are codominant and highly variable (Selkoe & Toonen, 2006). However, microsatellite data obtained through non-invasive samples has its shortcomings; for instance, the host DNA present in feces or hairs has lower quantity and quality than fresh tissues, which can lead to genotyping errors (Pompanon et al., 2005; Salgado-Lynn et al., 2016; Taberlet et al., 1999). The PCR procedure used to amplify this fragmented source of DNA can also be inhibited by the presence of other compounds present in the sample and can be degraded by its natural exposure to the environmental conditions (Taberlet et al., 1999). Consequently, during laboratory work, systematic errors such as "null alleles" can occur, where the PCR amplification of one allele systematically fails and causes incorrect assignment of a heterozygote as a homozygote. The same can occur with another main error – "allelic dropout" – where the poor quality/quantity of the sample can cause the amplification of one or both alleles at a given locus to fail. Finally, PCR artifacts or human errors in reading and/or recording data can lead to the mis-genotyping of one false allele as a true one (Johnson & Haydon, 2007; Pompanon et al., 2005).

Notwithstanding the difficulties involved in non-invasive sampling, it has been a valuable instrument to study several non-model threatened species and was readily added to the primate conservation toolbox,

having since been subjected to several strategies to minimize its limitations (Guichoux et al., 2011; Pompanon et al., 2005). The data made available through genetic analyses has informed on many primate species' demography, population dynamics, ecology, behavior and social structure, among other populational data (Di Fiore, 2003; Ferreira da Silva et al., 2012). Some findings made possible only through genetics were relevant for conservation planning, such as in the case of the Cross River Gorilla (*Gorilla gorilla diehli*). The genetic studies of the subpopulations of these animals suggest that they are actually a single, more-or-less continuous population, and that migration between the three identified subpopulations was still possible (Bergl et al., 2008; Bergl & Vigilant, 2006). This way, the genetic evidence informed on the conservation units to be considered, the location and direction of the migrations, confirming links in the gorilla habitat; in the future, these data can be valuable for demographic modeling analyses and establishment of effective habitat corridors (Oates et al., 2007). However, this strategic planning is not useful if the threats to primates are not, simultaneously, halted. The fragmentation and habitat loss that has been occurring in tropical forests not only reduces biodiversity, but also limits essential resources and habitat extension for wildlife. Eventually, this process reduces the population size and connectivity in most primate species, which can eventually lead to isolation in small forest patches (Dyke & Lamb, 2020; Estrada et al., 2012; Gibbons & Harcourt, 2009) and loss of genetic diversity within and among populations (Frankham et al., 2010; Minhós et al., 2016). The isolation of primates in the forest mosaic is additionally worsened not only due to the distance between forest patches (Gibbons & Harcourt, 2009), but also because of their reduction in quality (Galán-Acedo e t a l., 2 019). F inally, s ynergies b etween t he d ifferent t hreats s uch a s infrastructure (Ascensão et a l., 2 022) a nd a gricultural e xpansion (Estrada & G arber, 2 022), a s w ell a s a higher detection of the animals by hunters following the fragmentation and destruction of their habitat further endanger their populations (Estrada et al., 2012; Oates, 1996). Gene-based adaptations are unlikely to happen in time to protect primates from their habitat's changes, mainly due to their slow life-histories, long generation and reproduction rates (Kalbitzer & Chapman, 2018). Thus, although genetics cannot directly save these species in time, it can provide us with information on the species' characteristics that aid in conservation planning (DeSalle & Amato, 2004; Whiteley et al., 2015), through the measures of population genetics such as genetic variation, gene flow, effective population size, levels of inbreeding, population structure and demographic history (Allendorf et al., 2010).

1.7 Informative parameters in population genetics

1.7.1 Genetic Diversity

Habitat destruction and fragmentation frequently reduces the population sizes of organisms, which in turn often reduces genetic diversity and evolutionary potential of wildlife species (Frankham, 1996). The marked reduction in a population – a bottleneck – often results in the decline of gene flow between populations, which in many cases is already a problem due to the fragmentation of the forest. The lack of genetic exchange between populations paves the way for inbreeding depression, where the continuous reproduction between related individuals leads to a discernible decline in the "fitness" of the resulting offspring (Ascensão et al., 2022; Bergl et al., 2008; Dyke & Lamb, 2020; Pusey & Wolf, 1996). More specifically, a population that shows an elevated rate of inbreeding quickly loses genetic diversity, making it more prone to the nefarious effects that genetic drift has on a small population: fast loss of genetic diversity, fixation of deleterious alleles (and extinction of rare and new alleles) and population homogenization. Consequently, mutation (the source of all genetic diversity) is hampered, genetic drift effects are worsened, and natural selection is less efficient in such a small population (Frankham et al., 2010). Thus, an inbred population shows a decrease in its evolutionary potential, with the lessening of genetic diversity being proportional to the number of generations it remains small (Reed & Frankham, 2003). Paired with stochastic (Smith & Almeida, 2020) and demographic events such as bottlenecks

(Groombridge et al., 2000), the probability of a demographic collapse increases, and the population may become non-viable – rendering it functionally extinct. Every lost population equals less genetic diversity for the species and lesser capabilities for adaptation to environmental pressures, which can jeopardize its existence (Brooks et al., 2002; Frankham et al., 2010). In the case of primates, the problem of inbreeding depression has been documented in a meta-analysis, where several consequences of inbreeding were identified – ranging from 100% of potentially inbred offspring mortality within 30 days in wild yellow baboons (*Papio cynocephalus*), to lower birth weight of inbred offspring in captive rhesus monkeys (*Macaca mulatta*) and to the lighter and smaller inbred females of semi-free ranging mandrills (*Mandrillus sphinx*) that give birth earlier, among other examples (Charpentier et al., 2007).

There are established statistics which can illuminate a species or population's diversity, or the amount of genetic variability in its genetic pool. These mainly use the allele count and frequency across loci and individuals to measure genetic diversity in a series of ways; for example, we can sum the total number of alleles (N_a) and estimate the effective number of alleles (N_e) (the number of alleles needed to provide the same heterozygosity if all alleles were equally frequent). Heterozygosity levels are also informative on diversity, especially when we compare the observed (H_o) and expected (H_e) heterozygosities. In other words, the proportion of heterozygotes in a population, averaged over loci (H_o) against the estimation of that fraction based on known allelic frequencies (H_e). Allelic richness (A_r) measures not only the number of alleles per locus, but also their frequencies – which means it also considers the total number of samples (Frankham et al., 2010). The inbreeding coefficient (F_{is}) provides an estimation on the levels of inbreeding in the studied subpopulation (Wright, 1965). These and other measures of genetic diversity define the status of the sampled population, which is, as we have seen, determined by selection, mutation, migration, and genetic drift. In large populations, these forces have little effect, which is the contrary to what is observed in small populations. This is where the Hardy-Weinberg Equilibrium (HWE) can inform us on the status of the studied population, by providing an expected amount of equilibrium in allele and genotype frequencies in an imagined, large and panmictic population where there is no mutation, selection, or migration. Tested through the chi-square test, the significant difference between the expected and observed genotype frequencies (in other words, deviations from this equilibrium) can illuminate us on the levels of inbreeding or outbreeding of the population. If observed heterozygosity is significantly smaller than expected heterozygosity, we can assume that the studied population has a reduced genetic flow, meaning that it is fragmented and inbred; if the contrary is true, we can assume outbreeding, where genetically dissimilar individuals are able to mate (Stark, 2005; Waples, 2015). Finally, Linkage Disequilibrium (LD) – the non-random association of alleles at distinct loci – was also tested here, as it may indicate the effects of chance events, population bottlenecks, recent genetic exchange between different populations, inbreeding and selection on the associations among loci (Slatkin, 2008; Waples, 2015). Although these measures are valuable by themselves, the interpretation of their results will be paired with other analyses to provide a better illustration on the situation of the studied populations (Teixeira & Huber, 2021).

1.7.2 Relatedness

The study of familiar relations of social animals is essential to understand many individuals' behaviors, as higher cooperation would be expected to be recorded between close kin due to the benefits of maximizing inclusive fitness (Gardner & West, 2014; Hamilton, 1963). Before the development of molecular techniques, the study of relatedness between primates in a social group was a long-term task, restricted to mother-offspring relations (Queller & Goodnight, 1989). For instance, an observational study that focused for 26 years on a group of chimpanzees (*Pan troglodytes*) in Bossou, Guinea, revealed a different social organization from other chimpanzee groups, breaking the usual pattern of male philopatry and insistence on kin-related male bonds. The author suggests that this abnormal behavior in the chimpanzee context may be due to several possibilities such as a lack of conspecific competitors from neighboring groups, absence of predators, or even the shortage of medium-sized mammal prey to

hunt in groups (Sugiyama, 2004). Currently, the conjoined studies of molecular and observational data are especially helpful to understand the behavior of some species of primates. For example, a study on *Colobus guereza* groups in Kibale National Park described rare observations of female dispersal, but also presented conflicting molecular data that revealed numerous pairs of closely related adult females among the neighboring groups. The same authors mentioned four females that stayed in their natal group when their fathers and brothers were the only available mating partners. They didn't mate neither within nor outside their group, which the authors suggested might be due to the cost of dispersal to other groups of unknown kinship, or to augment their inclusive fitness, or even to help defend their group's feeding areas (Harris et al., 2009). Thus, the identification of kinship within groups of social animals is essential to unravel the evolution of their social systems and, consequently, help researchers understand the drivers behind some behaviors of the animals they are trying to conserve.
Scientists can accurately quantify relatedness in wild populations, between individuals of previously unknown relationship, especially with highly variable markers such as microsatellites. They do so through the calculation of a relatedness coefficient, or the probability that two individuals share an allele that is identical by descent (Kalinowski et al., 2006). These coefficients can be computed using linear regression (Lynch & Ritland, 1999; Queller & Goodnight, 1989; Wang, 2002) or maximum likelihood (Anderson & Weir, 2007; Kalinowski et al., 2006) methods, or even a combination of different estimators that can be joined with a spatial structure analysis (Kraemer & Gerlach, 2017). Various estimators usually give results ranging from -1, or less related than expected by chance, to 1, meaning more related than expected at random. The coefficient of relatedness used here is defined as the probability that two individuals share an allele that is identical by descent through the maternal (Rm) or paternal (Rp) lines. Thus, individuals sharing the maternal and paternal line (or full siblings) will share an Rm and Rp of 0.5, as will parent-offspring dyads (either Rm or Rp = 0.5). On the other hand, identical individuals (identical twins) will present a probability of Rm and Rp = 1, while half-siblings are expected to share 0.25 of their genomes (Queller & Goodnight, 1989).
The estimation of relatedness levels between individuals and/or populations is an essential step right at the initial stage of a genetic study, as their elevated concentrations may create biases in following analyses and their interpretations. For example, it will change our measurements of genetic diversity due to unexpected levels of similarity than what is expected in a more panmictic population, affecting the measures of heterozygosity and the tests that depend on it (Bergl et al., 2008; Rodriguez-Ramilo & Wang, 2012). Relatedness also has effect on dispersal analyses, as the average genetic relatedness of adults of the philopatric sex may be greater than between adults of the dispersing sex (Di Fiore, 2003). These considerations underscore the importance of understanding relatedness in genetic studies, as it can influence the outcomes and conclusions drawn from such investigations.

1.7.3 Structure

The genetic structure, or substructure in a population refers to the patterns of distribution of genetic diversity within a sampled population or among (sub)populations (Di Fiore, 2003). The genetic structure indicates the degree of differentiation within or between populations and informs us on its capacity to disperse throughout the habitat (Chikhi & Bruford, 2005). Classical studies of the population genetic subdivision and organization used Wright's F-statistics to summarize the effects of nonrandom mating within subpopulations on average individual heterozygosity (Wright, 1951). However, this classical model would incorporate individual-level effects on population divergence only indirectly, which is the reason for many improvements on structure detection methodology along the years (Falush et al., 2003; Legendre & Fortin, 2010; Peakall & Smouse, 2012; Pritchard et al., 2000). The mating system determines the genetic flow of the population, and so do barriers to dispersal of individuals (Di Fiore, 2003). Genetic drift causes stochastic loss of genetic diversity and has more effect in small populations, so while it decreases genetic diversity more at the subpopulation level, it will also increase genetic differentiation among the different populations. The constraints that lead to substructuring may also be

historical events, topographic features, habitat preferences, but also naturally and/or anthropogenically fragmented habitat, man-made infrastructures and explorations and even human presence and disturbance (Aleixo-Pais et al., 2019; Basto et al., 2016; Radespiel & Bruford, 2014). For example, the Cross River gorilla (*Gorilla gorilla diehli*) has been subject of a genetic study that illustrated the structure in the populations, but also detected migrants and individuals of admixed ancestry, providing encouraging results to the teams working on their conservation (Bergl & Vigilant, 2006). An investigation on another primate, the Guinea baboon (*Papio papio*) showed that the genetic discontinuities were not a product of anthropogenic dispersal barriers, nor geographical distance or habitat type. Instead, the authors proposed hunting pressure as the molder of the population structure, increasing dispersal distances and promulgating contact of previously separated socio-genetic groups (Ferreira da Silva et al., 2014). Therefore, the study of structure in primate populations is widespread and essential when considering conservation planning.

Microsatellites have been efficient in studies that assess genetic structure and several methods have been developed to infer structure through individual-based statistical methods. The verification of a population's structure is essential to not only have some clues on its genetic makeup, but also to inform other analyses that can be biased by its presence (Chikhi et al., 2010). However, these inferences have many difficulties in the mathematical, methodological, and biological sense (animals' behavior, social system, lifespan, dispersal and mating patterns, group formation processes, etc.) (Di Fiore, 2003; Evanno et al., 2005; Hoffman et al., 2017; Meirmans, 2015). Therefore, several methods to estimate substructure were used here, and eventually the geographical data were also added to the analyses to help obtain the most reliable and biologically relevant results.

1.7.4 Spatial genetic structure

Habitat continuity, as the many examples here provided indicate, is an essential set for the biodiversity orchestra in an ecosystem. Thus, the addition of the spatial background into molecular population genetic studies has been a relevant advance, as it contextualizes the species and/or population within its environment. Hence, the genetic data are analyzed with the information on the landscape features, which can greatly impact the distribution, movement, and overall behavior of organisms (Manel et al., 2003). This approach to the study of the way geographical and environmental characteristics come into play in genetic variation at the populational and individual levels has the advantage of not requiring previous knowledge on discrete populations (Manel et al., 2003; Segelbacher et al., 2010). This has important implications to the study of the ecology, evolution, and conservation of organisms, especially considering the novelty, swiftness and scale of the changes brought upon wildlife by humans.

Landscape genetics is a promising approach that continues to be improved and developed, as the complex interaction between genetic patterns of species and their habitat are continuously illuminated (Gruber & Adamack, 2015; Peterman, 2018). Its methodologies include analyses based on pairwise relatedness and on Bayesian methods, as well as inferences from landscape resistance maps (Segelbacher et al., 2010). I will focus on the first and second type of methodology, represented by spatial autocorrelation, isolation-by-distance (IBD) and Bayesian clustering. Spatial autocorrelation is widely used in genetic studies that seek to compare the relatedness of pairs of individuals with their respective geographical distances, which includes checking for a dependence of the genotype of an individual on the genotype of a neighboring individual (Manel et al., 2003). Thus, a deviation from zero in this relationship indicates that individuals at that distance class are more (positive values) or less (negative values) related than expected at random, which suggests spatial genetic structure (Peakall et al., 2003; Peakall & Smouse, 20). The distance classes are pre-defined by the researcher, which allows different class testing but also makes the detection of specifically localized discontinuities impossible (Manel et al., 2003).

In the context of a homogenous landscape, genetic differentiation between individuals that increases exclusively due to geographic distance resulting from local, geographically restricted dispersal is the

process known as isolation-by-distance (Wright, 1943). The verification of this pattern typically includes applying a Mantel test on a matrix of pairwise genetic distances and another matrix containing pairwise geographical distances (Guillot et al., 2009). When genetic drift and gene flow are in equilibrium in a population, we can predict a positive correlation between the two matrices; in other words, genetic differentiation between demes increases with geographical distance, indicating an IBD pattern (Meirmans, 2015). Conversely, the presence of barriers to dispersal in populations that would be otherwise panmictic can also indicate a positive correlation, which has a different origin in such cases (Guillot et al., 2009). Thus, great care must be taken when analyzing IBD patterns, as they may be biased by structure and by the very model that confirms the presence of IBD (Meirmans, 2012). On the other hand, when neighboring groups but not distant groups have higher than expected relatedness (Hutchison & Templeton, 1999; Peakall et al., 2003), a disruption of the IBD pattern is observed. There are also different possible origins of this genetic configuration through the landscape: it may be due to the presence of a barrier (at the landscape or at the species' level) that impedes dispersal throughout the landscape (Hoffman et al., 2017) or even an extensive degree of dispersal (Ehrich & Stenseth, 2001). Bayesian clustering methodologies – the second type used here – use multilocus genotypes to cluster individuals into populations that minimize HWE and LD. Using a Markov chain Monte Carlo (MCMC) algorithm, they assign each individual to a cluster where its posterior probability of membership is the highest. When genetic discontinuities are detected in the landscape, the dataset is divided into subpopulations to maximize equilibrium (Chen et al., 2007; François et al., 2006; Manel et al., 2003; Pritchard et al., 2000). Although these methods provide robust results, there is a possibility that these analyses can create false clustering of individuals due to the various factors that can be read as structure (such as bottlenecks, inbreeding, admixture and reduced populations) (Manel et al., 2003). Additionally, they can also fail to identify true genetic boundaries in presence of a strong isolation-by-distance pattern (Safner et al., 2011).

This approach has proved itself valuable in conservation, as in the case of the marsh grasshopper (*Stethophyma grossum*), where the consideration of genetic flow of this species in different scenarios of landscape configurations aided in strategic landscape conservation planning (van Strien et al., 2014). Several different Bayesian algorithms have also proved its use in a study of two widespread carnivorous species (*Martes foina* and *Vulpes vulpes*) in Portugal, illustrating their populations' structure and spatial boundaries (Basto et al., 2016). In the case of primates, a study on a mouse lemur (*Microcebus tavaratra*) using microsatellites clarified the impact of an open habitat, type of vegetation and a river on the animals' population structure. The authors used landscape genetics tools to show that this species maintains substantial levels of genetic diversity at the forest patch and population level, but not between forest patches, thus illustrating the effects of habitat fragmentation on the species (Aleixo-Pais et al., 2019). Hence, it is essential to study primates continuously in the dynamic context in which they inhabit, as anthropogenic habitat change can alter their populations and species status quite quickly. Therefore, conservation efforts must become more holistic in terms of primate and forest ecology, as well as include human considerations.

1.7.5 Sex-biased dispersal

Dispersal – the movement of individuals from their natal groups to establish in other locations for breeding – is critical to a species' survival prospects, as it affects gene flow and diversity directly (Saastamoinen et al., 2018). More specifically, it counteracts the effects of genetic drift through the maintenance of connections between populations and subpopulations, which prevents their isolation (Jones, 2003). Thus, it has dramatic consequences on the genetic makeup of populations, with complex interactions between a species' mating system, dispersal abilities, inbreeding avoidance, as well as kin cooperation and competition. The degree of fragmentation of habitat also influences the organisms' dispersal abilities, as well as their success, or lack thereof, in adapting to this kind of landscape. Although these complex interactions were difficult to study in the past, nowadays, population genetics studies

account for the social structures of populations and variations in individuals' behaviors when describing gene flow. This is especially important for social animals such as primates, as understanding their (usually skewed) dispersal patterns helps unravel the complexity and evolution of their social systems (Di Fiore, 2003). The main underlying reasons for this behavioral pattern have been pointed out to be strategies of inbreeding avoidance, intrasexual competition for mates and intragroup competition for resources – all of which are traits related to group-living, social animals (Fields & Guatelli-Steinberg, 2003). Another possibility is avoidance of aggression and/or infanticide, which has been hypothesized to be the origin of the high rates of female dispersal in the Tana River red colobus (*Piliocolobus badius rufomitratus*) (Marsh, 1979). During the three years of studying these colobines in Wenje (Eastern Kenya), researchers noted that males had a high rate of turnover through replacement, after which rates of infant death would increase. As a result, it would be logical for females to travel to neighboring groups and assess a male's ability to hold a harem for the time needed for them to get pregnant and rear an infant, before becoming residents in the group.

When dispersal is sex-biased, a difference is genetic structure is expected between the nuclear genetic markers (inherited both maternally and paternally) and the mitochondrial (for females) or Y-linked genes (for males) (Avise, 1995). Generally, in primates and other social animals, dispersal is biased toward the male side, although there are many examples of different dispersal patterns (Moore, 1992). Species where females exhibit philopatry and where males are the dispersing sex are expected to present substructuring in their mitochondrial genes and little to no genetic substructuring in the Y-linked genes. However, species characterized by female dispersal are expected to present equivalent levels of substructuring to mitochondrial and nuclear diversity, as female gene flow homogenizes both genomes across populations. As for species where both sexes disperse, as in the case of some colobines, comparable levels of population substructure are also expected in both genomes (Avise, 1995). The direction of instantaneous sex-biased dispersal (meaning dispersal in one generation) can be standardly assessed through the levels of genetic structure of females and males in a population with parameters like the fixation index (Fst), relatedness (r) and inbreeding coefficient (F_{is}) (Goudet et al., 2002; Weir & Cockerham, 1984; Whitlock & McCauley, 1999) or through the calculation of the probability of an individual having its origin in the population from where it was sampled by calculating the mean ($m\text{AI}_c$) and variance ($v\text{AI}_c$) of the corrected assignment index (Goudet et al., 2002; Lawson, Handley & Perrin, 2007). The detection of sex bias in dispersal will depend on the intensity of the bias, as well as the rate of the dispersal (Goudet et al., 2002). If the bias intensity is low, only Fst and $m\text{AI}_c$ methodologies are able to detect it, while $m\text{AI}_c$ is not too sensitive to both the presence of rare alleles and to a population mainly composed of dispersers. Also, one must take into account the weakness of these tests when they are performed in isolation, as the sampling design and the number and variability of the chosen loci can influence the detectability of sex-biased dispersal (Lawson, Handley & Perrin, 2007). Finally, in the case of this study, the tests were conducted knowing the sex of individuals but not their age, which means that both pre-dispersing (usually infants and juveniles) and post-dispersing (usually adults) were included. Although this analysis may be useful, the presence of the pre-dispersing individuals can mask the sex inclination in dispersal (Lawson Handley & Perrin, 2007).

1.7.6 Demographic History

The detection of important demographic events in a species' history is essential to understand its populations' past and current state, as well as provide clues for its future distribution. These leave traces in the genetic signature of the animals, which can be detected through distributions of allele size in microsatellite loci (Beaumont, 1999; Cornuet & Luikart, 1996; Goossens et al., 2006). The detection of serious and sudden declines or expansions in a species' past can help us understand the factors that led to these changes (biotic or abiotic, natural, or artificial) and possibly inform on the resilience capabilities of organisms when facing analogous threats in the future (Goossens et al., 2005; Minhós et al., 2016; Quéméré et al., 2012). For instance, the demographic collapse of the Bornean orangutan (*Pongo*

pygmaeus) was detected with genetic data, where demographic history helped illuminate on the impacts that anthropogenic fragmentation and deforestation were having on this great ape (Goossens et al., 2006). Another study looked at the possibility of a simultaneous population expansion in savannah baboons (*Papio cynocephalus*) during the African human and chimpanzee late Pleistocene population expansion. The results indicated an absence of a shared population expansion of the baboons, joining another piece to the puzzle of human and non-human primate evolutionary history (Storz et al., 2002). However, one must take great care in the study of demographic history, since there are different factors that can lead to erroneous conclusions on population changes. For example, biased sampling schemes (Radespiel & Bruford, 2014) and substructure can induce a fake signal of population decline, leading to an incorrect assumption of a past bottleneck (Chikhi et al., 2010). Fortunately, methods for these kinds of inferences have seen a great level of diversity and improvements, which augments our chances of conducting successful studies (Garza & Williamson, 2001; Girod et al., 2011). For example, sampling can be performed on several subpopulations and/or fragments; therefore, if the bottleneck signal is detected when gathering samples from several demes, we can more safely assume that there has been a change in the size of the metapopulation. Another possible solution is, instead of using different samples in the spatial realm, temporally distinct samples (present and past DNA) can be used to distinguish patterns of population change and structure. Additionally, comparing real data with simulation data may be helpful to confirm whether the substructures in the real population are responsible for the resulting genetic patterns (Chikhi et al., 2010). Finally, it is important to take into account the assumptions behind models that are used as bases when testing for population size changes (such as the Wright-Fisher model, which assumes panmixia and demographic stationarity) as well as the predetermined parameters of the test when looking at the results of the specific populations under study (Chikhi et al., 2010; Girod et al., 2011).

1.8 Importance, objectives, and hypothesis

Colobines represent, according to recent data (Fernández et al., 2022), one of the most endangered taxonomic groups of primates, considering the number of major threats they face. Colobus monkeys are known for their high levels of dependence on the forest habitat, which is being fragmented and disappearing swiftly. Furthermore, although their populations in Sierra Leone have been surveyed fairly recently, there have been no genetic studies conducted on the red and black-and-white colobus. Hence, we lack knowledge on their population connectivity, genetic diversity, inbreeding levels and demographic history which might be fundamental for effectively managing and protecting their populations. What is known of their populations in Sierra Leone is that both colobus monkeys are now rare and patchily distributed (Brncic et al., 2010; McGraw et al., 2020) and that GRNP may be a refugium for these species in the country. Therefore, this genetic study will be providing novel genetic data of the populations of *C. polykomos* and *P. b. badius* in Gola Rainforest National Park and the Tiwai island, to improve the knowledge of their populations' status in the protected areas. Our team is currently surveying other areas in Sierra Leone which, together with the results from this thesis, will complement our understanding of the population dynamics and demography of the colobine monkeys across the country.

More specifically, the aims of the present study are to:

I. Inform on the genetic diversity and inbreeding levels of the populations of *C. polykomos* and *P. b. badius*.
II. Describe the colobines' patterns of genetic structure at the populational and social group level.
III. Understand how the habitat matrix shapes their genetic diversity.

IV. Investigate the existence of a sex-biased dispersal.
V. Unravel the demographic history of these colobines.

Given the demonstrated significance of preserving large and continuous forests for maintaining the genetic condition and evolutionary potential of red and black-and-white colobus populations (Minhós et al., 2023), this study seeks to explore the following research questions within the large and continuous forests of GRNP:

1. What are the levels of genetic diversity observed in these populations?
2. To what extent does population connectivity exist without major genetic differentiation?
3. How is genetic diversity distributed across the landscape, and does it conform to an isolation-by-distance pattern?
4. What patterns of sex-biased dispersal, if any, emerge within these cohesive habitats, particularly due to the lack of significant disturbances in the protected area?
5. Can these data uncover the size and stability of the historic and current effective populations?

Due to the high dependence of these primates on the forest, I expect that the results yielded by the present study will show a relatively positive portrayal of their populations in the GRNP and Tiwai. Hopefully, this study will contribute contemporary information on these two threatened West African primates, providing valuable data to inform on the conservation of both species across their distribution. Finally, I hope that the data presented here can add value to the current knowledge on *C. polykomos* and *P. b. badius* and contribute to protect the Gola landscape.

2 Methods

2.1 Study area

Gola Rainforest National Park is located in the southeast of Sierra Leone (between latitude 07°18'22" N and 07°51'00" N, and between longitude 11°21'13" W to 10°37'40" W), at the border with Liberia. Its area of 710,7 km² is subdivided into three forest blocks: Gola North, Gola Central, and Gola South. This study includes the (~12 km ²) Tiwai island, situated at the northwest of the GRNP (Figure 2.1). This island is 5 km away from Gola South and it is surrounded by a large river (Moa). The main rivers of the park are Magbole, located in Gola North, Mogbai in Gola Central, and the Mahoi river that crosses Gola South, which are filled with 2,500 to 3,000 mm of annual rainfall, mostly between July and August (Bergl et al., 2008; Dyke & Lamb, 2020). The Gola North block has the highest altitudes (79% of the area with over 250 m) and, due to this difficult terrain, the forests in the northern and eastern parts of the block were never commercially exploited (Klop et al., 2008). It is included in the Kailahun district, along with a part of Gola Central, which continues on to the Kenema district that comprises Tiwai and Gola South (the rest of this latter block being in the Pujehun district) (Bulte, et al., 2013).

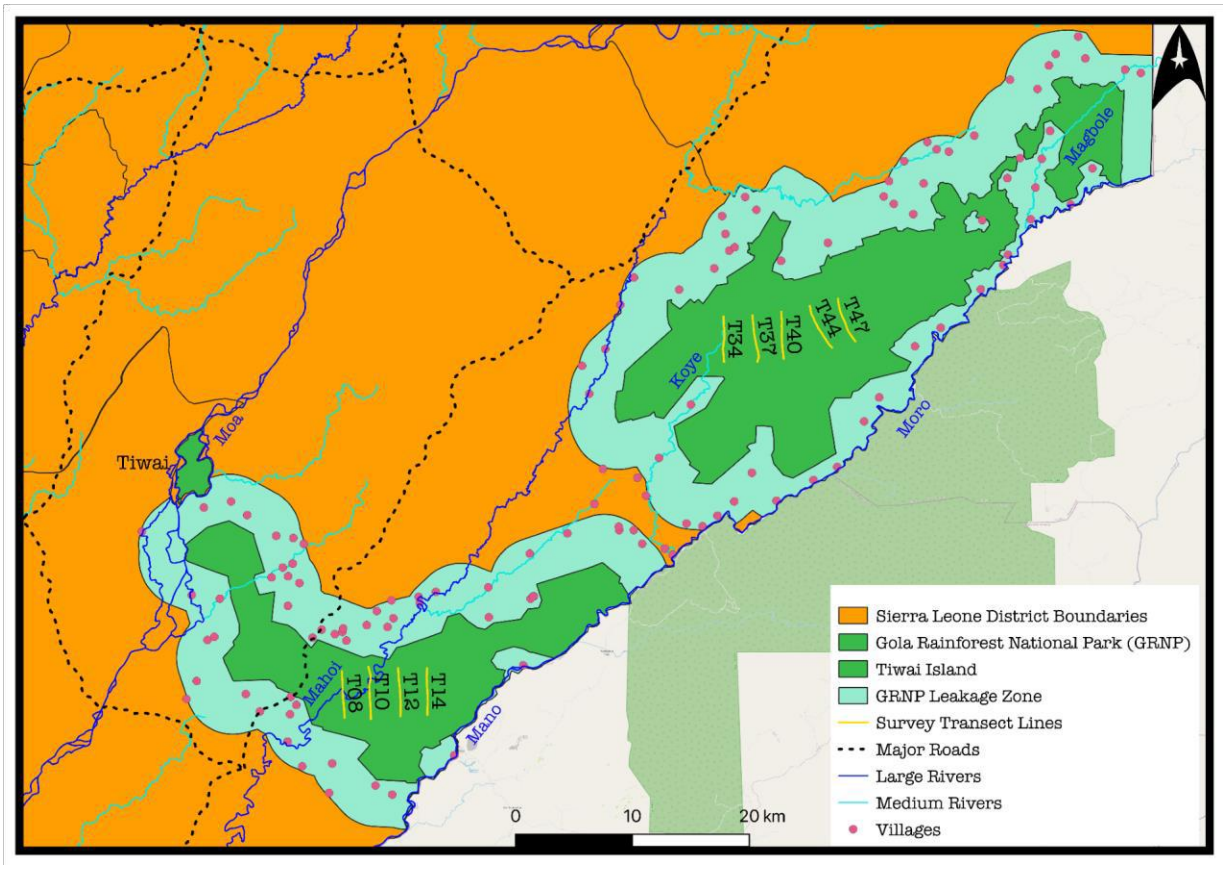


Figure 2.1 Detailed map of the border between Sierra Leone (orange) and Liberia (white with green in Gola region), where one can see the Tiwai island, Gola Rainforest National Park and respective leakage zone. Here, the survey transect lines and names can be observed, as well as landscape features such as major roads, large and medium rivers and villages. Produced in QuantumGis v. 3.16.10-Hannover.

The protected area is Sierra Leone's remaining tract of lowland moist evergreen high forests, and it lies at the western extremity of the Upper Guinean Forests subregion of the Guinean Forests of West Africa hotspot, which contains a great level of species richness and endemisms (IUCN, 2015; Lindsell et al., 2011; Lindsell & Klop, 2013). Its forests started being exploited commercially for timber in the 1960's mainly in the Central and South Blocks, with conservation works starting here in 1989. This effort was fundamental to preserve unique species found here, such as the Pygmy hippopotamus (*Hexaprotodon liberiensis*), the Jentink's duiker (*Cephalophus jentinki*) the African Forest Elephant (*Loxodonta cyclotis*), the white-necked Picathartes (*Picathartes gymnocephalus*) and the Taï toad (*Amietophrynus taiensis*). This is also the home of many primate species, such as the Demidoff's dwarf galago (*Galagoides demidovii*), the Thomas's dwarf galago (*Galagoides thomasi*), the Potto (*Perodicticus potto*), the Sooty mangabey (*Cercocebus atys*), the Diana monkey (*Cercopithecus diana*) the Olive colobus (*Procolobus verus*), the Campbell's monkey (*Cercopithecus campbelli*), the Lesser spot-nosed monkey (*Cercopithecus petaurista*), the Green monkey (*Chlorocebus sabaeus*) and the western Chimpanzee (*Pan troglodytes verus*). Finally, we can also find in GRNP the taxa presented in this study: the western black-and-white or King colobus (*Colobus polykomos*) and the Bay colobus (*Piliocolobus badius badius*). The 1991-2001 Sierra Leone civil war did not seem to seriously impact the mammals that are found in GRNP (Lindsell et al., 2011). Nonetheless, one cannot dismiss indirect consequences that may still be found in the future (Dudley et al., 2002).

The GRNP was set up officially in 2011, through the Gola Forest Program (GFP) – a partnership between the government's Ministry of Agriculture, Forestry and Food security (MAFF), the Conservation Society of Sierra Leone (CSSL) and the Royal Society for the Protection of Birds (RSPBUK) (Government of Sierra Leone, 2017). This partnership is the base on which conservation work is conducted in the park,

with the enforcement of 50 guards and the communities that depend on the forests' ecosystem services (Crawford et al., 2011). The protected area is encircled by a 4 km-wide leakage belt, containing 122 human communities that are home to approximately 23 500 people (RSPB, 2015). Most of their subsistence (90%) is related to farming, with access to land being regulated for the inhabitants of the forests. Although they are allowed to harvest non-timber forest products (NTFPs), logging, mining and hunting activities are prohibited. Nevertheless, in practice these do still occur (Davies, 1987; Bulte et al., 2013).

2.2 Study species

This study focuses on two taxa: the western black-and-white colobus or King colobus (*Colobus polykomos*) and the Bay colobus (*Piliocolobus badius badius*). Both are classified as Endangered (EN) by the IUCN and present a decreasing trend in their populations' number (Gonedelé Bi et al., 2019; McGraw et al., 2020). Due to their high dependence on forest habitat, these arboreal species are good indicators of the ecosystem's overall status (Hillers & Tatum-Hume, 2013). These species, belonging to the Old-World subfamily *Colobinae*, exhibit some degree of similarity in their diet and ecology, occasionally coexisting in sympatry, as seen in the case of the GRNP (Minhós et al., 2013a). Both monkeys are specialists in their habitat and diet, rendering them particularly sensitive to significant habitat degradation (Minhós et al., 2016).

The Western Black-and-White colobus (further designated here as black-and-white colobus or BWC) inhabits rainforests and gallery forests in Guinea-Bissau, Guinea, Ivory Coast, Liberia, and Sierra Leone (Gonedelé Bi et al., 2019). Their diet consists of seeds, young leaves, and occasional flowers when these preferred foods are abundant. During times of food scarcity, they adapt by incorporating old leaves into their diet (Davies et al., 1999). Despite their strong dependence on forest habitats, they've demonstrated a degree of resilience in the face of habitat changes, such as inhabiting smaller forest patches, adjusting group size and dispersion, and modifying their diets (Chapman et al., 2000; Minhós et al., 2016). Typically, these monkeys form groups comprising 10 to 15 individuals. Males are the dispersing sex, while females exhibit philopatry (Minhós et al., 2013a). Unfortunately, their populations face the threat of hunting across their range, compounded by the ongoing destruction, degradation, and fragmentation of their forest habitats, putting them at risk of disappearing (Gonedelé Bi et al., 2019; Oates, 1996). The animals' presence in Sierra Leone has not been studied extensively, although some studies have been conducted in GRNP (Dasilva, 1992; Davies, 1987; Davies et al., 1999; Klop et al., 2008; Lindsell et al., 2011). The most recent surveys found 24 groups, situating their group densities at around 1.31 individuals/km² with a 95% confidence interval between 0.89 and 1.94 (Lindsell et al., 2011). The same study estimated that there are 8,876 individuals in the Gola forests, a considerable decrease from previous surveys. Although they were observed in primary and logged forest, their presence was stronger in the unlogged forests of Gola North and Tiwai (Klop et al., 2008).

The Bay colobus (*Piliocolobus badius badius*) (further designated here as red colobus or RC) is a subspecies that can be found in southern Guinea, Ivory Coast, Liberia, and Sierra Leone, though in the latter country it may be found beside the other subspecies in the North (Linder et al., 2021). The animals can be found in several forest types, such as primary, secondary, and gallery forests, woodland, tree and shrub savannas and mangrove formations (McGraw et al., 2020b). The monkeys have a clear preference for old growth forests, where they thrive on a diet of fruit, seeds, young leaves, and flowers, also adding mature leaves during the late wet season (Davies et al., 1999; Lindsell et al., 2011). This is the case in Gola, where the animals have been recorded most frequently in the Gola North block with an estimated population density of 30.5 animals/km² (Klop et al., 2008). They gather in multi-male and multi-female groups ranging from 2 to 60 individuals, with dispersal usually being mediated by females (Minhós et al., 2013a; Struhsaker, 2010). They are hunted for subsistence and bushmeat trade throughout their range of distribution, this being one of their main threats (Linder et al., 2021). Deforestation due to logging,

mining, charcoal production, and farming, which have been growing concomitantly with the human population, represent the other threats to the animals' survival (Linder et al., 2021). Their presence has been diminished after the civil war (although no direct evidence has pointed to the war as the origin of that decrease, for more details see Lindsell et al., 2011) and their total numbers have been estimated to be around 14,831 individuals (Klop et al., 2008). A recent survey recorded 16 groups in the park, situating their group density at about 0.87/km² with a 95% confidence interval between 0.52 and 1.47 (Lindsell et al., 2011). Some studies have shown that these animals are extremely sensitive to habitat alterations, that if too great, can pressure the monkeys to disappear (Linder et al., 2021; Minhós et al., 2016). They require a large variety of plant species to meet their dietary requirements, probably due to their folivorous adaptations, but they have also presented some degree of flexibility in the use of different species of plants and plant parts for food, which gives reason for hope in populations that live in altered habitats (Aleixo-Pais, 2022; Chapman et al., 2002).

2.3 Data collection

The fieldwork necessary to collect the non-invasive fecal samples and their geographic locations was conducted in 2018 by a team led by Isa Aleixo-Pais and Filipa Borges, under the FCT-funded PRIMATOMICS research project (PTDC/IVC - ANT/3058/2014). Sampling was conducted using already available transects that are routinely used in various surveys conducted in this protected area (Klop et al., 2008). The colobus fecal samples were collected (Figure 2.2) whenever a colobus social group was spotted or heard, and only fresh fecal material was collected. The team of researchers used gloves and face masks to avoid contamination and prevent disease transmission when collecting the samples and stored them in falcon tubes containing silica gel. The team recorded all the relevant information for each individual fecal sample (putative species, date, location, collector, etc.) both in the collection falcon tube and field sheet. The GPS data of each sample was recorded with a Garmin GPS Map 64s device and uploaded to a cloud storage. The stools were preserved according to literature guidelines (Roeder et al., 2004) and shipped to Instituto Gulbenkian da Ciência (IGC). The genetic data was produced at the fully equipped Population and Conservation genetics group laboratory and at the Genomics Facility at IGC by the PhD candidate Filipa Borges. The "QIAamp Fast DNA Stool Mini Kit" from Qiagen was used for DNA extractions, following instructions from the manual with some modifications. The incubation occurred overnight at 56ºC in order to increase cellular lysis and the centrifugation and incubation periods were tripled (3 minutes and 30 minutes, respectively). Finally, the volume of elution was decreased to 100 µl to augment the final DNA concentration, incubating for 10 minutes and centrifuging at full speed for 3 minutes (Borges et al., in prep.).

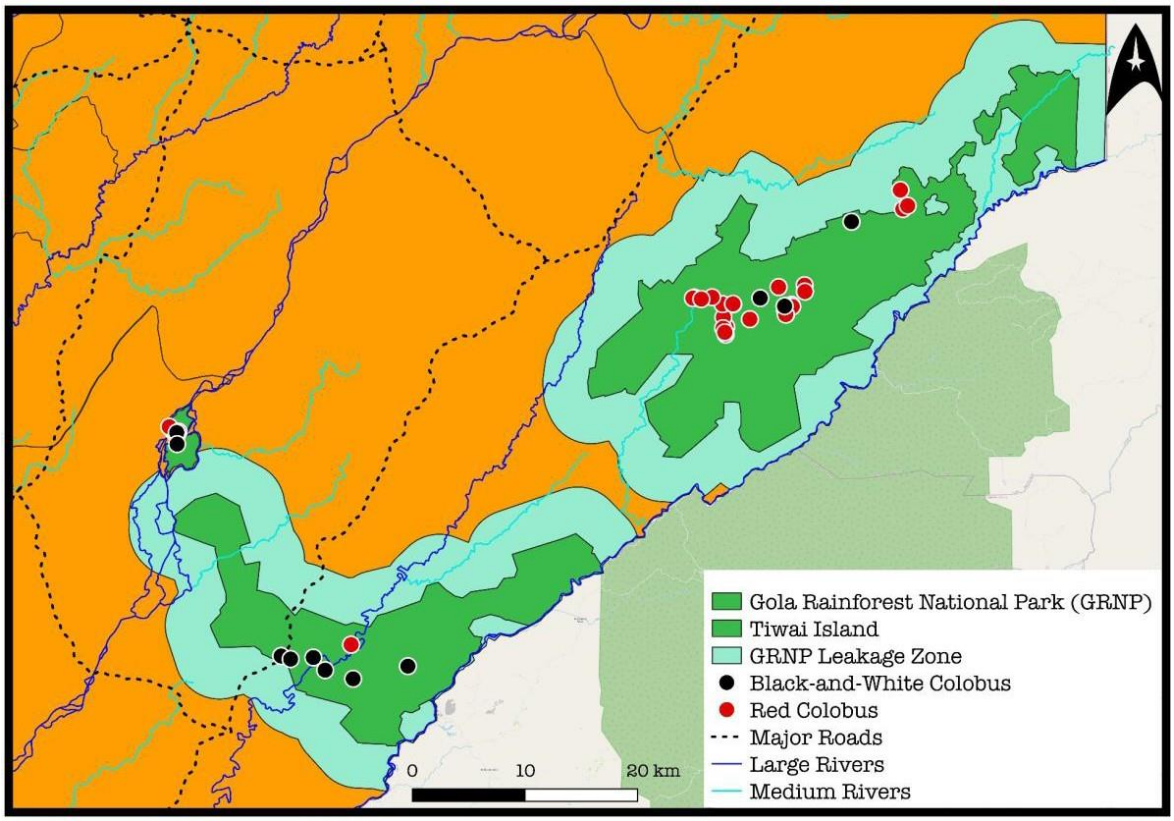


Figure 2.2 Detailed map with the Tiwai island, Gola Rainforest National Park and respective leakage zone. Here, beside the landscape features such as major roads, large and medium rivers, one can also observe the specific locations of the samples of the black-and-white (black circles) and red colobus (red circles). Produced in Quantum GIS v. 3.16.10-Hannover.

After DNA extraction, total DNA concentration was measured in a Thermo Fisher Scientific NanoDrop 2000 spectrophotometer. The molecular identification of the species was conducted by the amplification and sequencing of a 400-bp fragment of the D-loop region of mtDNA, followed by a comparison of the consensus sequences with those deposited in public online databases. To genotype the samples identified as corresponding to our two species of interest, 3 multiplex polymerase chain-reactions (PCRs) were conducted to amplify 15 microsatellite loci and one sex marker, and the results were uploaded and read with the program Peak Scanner 1.0 (Thermo Fisher Scientific). We conducted at least three independent amplifications of each locus to reach the consensus genotype and to control for the genotyping errors resulting from the amplification of the low quality and quantity fecal DNA. The output from Peak Scanner was transformed into two .xlsx files: one containing genetic information of the 54 black-and-white colobus samples and another with the data from the 198 samples of red colobus. Then, the 4-fase quality control analysis began: calculations of amplification success of the samples, verification of error rates, estimation of the consensus threshold and calculations of probability of identity, as explained below.

2.4 Quality Analyses

2.4.1 Quality Index

To ensure the best quality in the data at an early stage, there was a need to ascertain the quality of the genotypes, by identifying and discarding the ones that were of low quality. This control of the genotype quality is especially important when obtaining microsatellite data from fecal DNA that is usually of low quality and amount (He et al., 2011; Salgado-Lynn et al., 2016). Most of the target DNA is in small quantity (in the picogram range) compared to the rest of the matter (undigested food, digestive enzymes,

mucus, bile salts, bilirubin, and microorganisms), and it is concentrated in the outer lining and on the "front end" of the fecal material. Concomitantly, it is also subjected to UV light, tropical climatic conditions (high humidity and temperature) and presence of decomposers and chemical inhibitors of DNA extraction and amplification (Salgado-Lynn et al., 2016; Taberlet et al., 1999).
Following instructions for calculation of the Quality Index (QI) (Miquel et al., 2006), the number "1" was attributed to the repeats that corresponded to the preliminary consensus genotype and the number "0" to the ones that did not, irrespective of the reason. Each allele that appeared at least two times in different repeats was considered "true" for heterozygotes. To avoid the confounding effect of allelic dropout, a single allele had to be observed in at least two repeats for that individual to be considered a "true" homozygote for that marker. Also, in the cases where an equal number of two different genotypes was detected, "0" was attributed to that sample, since there was no certainty about neither of the options. The QI of each sample was calculated as the average score across several repeats, which could be up to 10 per sample in some cases, due to optimization exercises. Each locus also has an associated QI, calculated as the average QI across samples.

2.4.2 Estimation of False Alleles and Allelic Dropout

When low quantity/quality DNA is used (as in the case of non-invasive fecal sampling) errors associated with genotyping are expectable and must be detected in the samples and loci to ensure the best quality in the database. The most usual errors are false allele, when an amplification of a PCR produces an artifact, and allelic dropout, where one or both alleles at a given locus fail to amplify due to low sample quality. This can lead to the misidentification of a heterozygote as a homozygote due to the recognition of only one of two alleles (He et al., 2011).
To calculate the rates of allelic dropout and false alleles, the software GIMLET v. 1.3.3 (Valière, 2002) was used, considering that it has no limits to the number of repeats per sample, gives calculations for other types of errors besides allelic dropout and false alleles and supplies more details about the dataset. The only downside is that bigger databases (which is the case of RC) must be broken down (here it was divided into 3 databases) to be analyzed by the program. To confirm the output, the frequencies per allele were summed and divided by the sum of all frequencies, obtaining the mean relative frequencies of alleles for each locus.

2.4.3 Estimation of Consensus Threshold

Having the error rates calculated using GIMLET, we proceeded to the estimation of the allelic frequencies with the GenAlEx v. 6.5 (Peakall & Smouse, 2012) extension of Excel v. 16.58, to have all the necessary input for GEMINI v. 1.3.0 (Valière et al., 2002). This software determines the minimum number of times we need to see an allele per locus – or consensus threshold. With this information, the program estimates the minimum number of PCR repetitions across loci to warrant the highest degree of confidence in the genotypes. Since the maximum number of repeats in the databases was 10, the range of the repetitions number was set from 2 to 12 with 10 000 replicates. This way, it was possible to conclude that the two databases reached an asymptote at 4 replicates (97% confidence level for BWC and 99% for RC), which indicates that increasing the number of repetitions after 4 would not significantly improve the reliability of the genotypes. Therefore, a new QI was calculated using only 4 repetitions per sample in the cases where there were more and 3 when this was the available number of repetitions.

2.4.4 Detection of repeated individuals

Sampling the fecal material for DNA analyses, without direct observation of the animals, is a methodology prone to sampling the same individuals multiple times. Therefore, I used GenAlEx's Multilocus Matches, to detect samples that have a high degree of similarity and thus a high probability of belonging to the same

individual. The samples that were equivalent (equal in all genotypes across loci) were matched, ignoring the missing data. Among each pair of matching samples, the one with a lower QI and/or higher amount of missing data was deleted from the datasets.

2.4.5 Error and outlier detection

Since error detection is an essential process associated with the confirmation of the data before proceeding to analyses, another method was used for this purpose. This time, the focus was on errors related to the amplification and genotyping processes. The software MICRO-CHECKER v. 2.2.3. (Van Oosterhout et al., 2004) was used, conducting 10,000 runs for each dataset. Thus, it was possible to detect putative null alleles, large allelic dropout and scoring errors due to stuttering. The inconsistent values detected by the software were verified and the output of errors previously described was summarized by locus. Since the program detected a few problematic loci, the samples with 5 or more occasions of missing data were removed (to avoid biasing the results) and the tests were rerun. A preliminary analysis was conducted to identify departures from the HWE and the degree of LD, using Genepop on the Web v. 4.7.5. (Raymond & Rousset, 1995; Rousset, 2008), with a dememorization number of 10,000 for 1,000 batches and 10,000 iterations per batch in both tests. These informed the decision to remove more samples with missing data to ensure higher quality. Two more databases were created, so overall there were 3 datasets for each species: the "original", one without samples containing 5 or more occasions of missing data and another one without samples containing 3 or more occasions of missing data. The three tests previously described were rerun on these new datasets to test for the effect of the missing data and select the dataset that would ensure the highest number of samples with the highest quality possible.

Afterwards, GENETIX v. 4.05 (Belkhir et al., 1996) was used to conduct a Factorial Correspondence Analysis (FCA) with the six datasets created previously (three for each species) to detect any possible extreme values in the datasets, that could be the consequence of genotyping errors. The genotypes of the identified extreme values were checked for errors and missing data. Also, the HWE test was again conducted without these extreme values, to identify the samples that were altering our results to a greater extent. This way, it was possible to decide which samples would be deleted from the databases before moving on to the next analyses. Samples with a QI > 0.53 and less than five genotypes with missing data were maintained, leaving us with 35 samples for BWC and 160 samples for RC.

2.5 Genetic Diversity

The GenAlEx extension of Excel and R packages 'hierfstat' v. 0.5.7 (Goudet, 2005) and 'adegenet' v. 2.1.3 (Jombart, 2008) were used to estimate the genetic diversity through established parameters (Frankham et al., 2010): N_a (number of alleles), N_e (number of effective alleles), H_e (expected heterozygosity), H_o (observed heterozygosity), A_r (allelic richness) and F_{is} (inbreeding coefficient). The analyses with these parameters were conducted at first on the complete datasets with 15 loci (BWC) and 14 loci (RC). Additionally, final tests of LD and deviations from HWE were performed using the previously used specifications, and *p* values were adjusted through the Bonferroni correction to test for statistical significance throughout multiple simultaneous comparisons (in this case, of loci).

After testing for HWE deviations, presence of LD and of null alleles, it was decided that following analyses should be performed with and without some problematic loci (for details, see 3.1. Quality Control in the Results section). If the following analyses would be performed in this way, we could compare and observe any bias that resulted from the presence of loci with null alleles, with LD and deviations from HWE. Thus, the final datasets were created, containing 25 samples of BWC (one with 15 loci and another with 12) and 146 samples of RC (one with 14 loci and another with 12). The following analyses for each species were conducted with both datasets (same individuals with different number of loci) to decide which dataset would be considered for the Results section.

2.6 Relatedness

It is necessary to test for the presence of highly related individuals in the datasets, considering that the sampling of social groups can include related individuals − which may induce substructure (Pritchard et al., 2010). For this purpose, the software Kingroup v. 2 (Konovalov et al., 2004) was used with the Goodnight & Queller estimator (Goodnight & Queller, 1999) to test for relatedness of full siblings against unrelated individuals and parent-offspring against unrelated individuals. The maximum number of simulations of possible pairs allowed by the program was selected (100,000) and the pairs with a level of significance < 5% were identified. In other words, pairs of samples which have a p value < 0.05 were considered as significantly related at first (Konovalov et al., 2004). However, after performing a preliminary run with the STRUCTURE program, it was decided that the relatedness analysis wouldn't be necessary in the case of the RC, since this population did not present any substructure in the population. Thus, only the BWC population was subjected to this analysis, with a lower p value being considered with the objective of making the best compromise between data quality and sample number.

2.7 Populations' Structure

STRUCTURE v. 2.3.4. (Pritchard et al., 2000) was used to detect possible substructuring within the two populations, by calculating the probability of the individuals belonging to different genetic groups. The program has been widely used for this purpose and it still provides valuable input on the inference of genetic structure (Chen et al., 2007; Frankham et al., 2010). More specifically, this analysis can illustrate the level of differentiation between the individuals of each population, indicating the dispersal capabilities of the individuals through their habitat (Chikhi & Bruford, 2005).

The number of genetic clusters in a population − K − was set from 1 to 5, with 5 independent simulations per K, a burn-in period of 100,000 and 1,000,000 Markov Chain Monte Carlo (MCMC) sweeps (Falush et al., 2003). Afterwards, the output was uploaded to Structure Harvester v. 0.6.94 (Earl & vonHoldt, 2012) to confirm the interpretation of the results and to identify the most likely number of K given the data. Since this method identifies a minimum of two clusters (which may not be the case of our populations), the results were again confirmed with the estimation of the most probable number of clusters through the calculation of Posterior Probability (Pritchard et al., 2000).

STRUCTURE was run at first for the databases mentioned above (15 loci (BWC) and 14 loci (RC) and with 12 loci for both) to test for the substructuring. After finding family-induced structure for the BWC datasets, the highly related individuals were removed from the dataset to prevent false signals of population structure and population size changes, leaving us with 18 samples instead of 25. This became an additional dataset to use along with the complete one of 25 samples in analyses where related individuals could bias the results.

To further explore the data, a Principal Component Analysis (PCA) was conducted using RStudio v. 1.4.1106 (RStudio Team, 2021), with the packages 'adegenet' v. 2.1.3 (Jombart, 2008), 'ade4' v. 1.7.16 (Chessel et al., 2004; Dray & Dufour, 2007), 'PopGenReport' v. 3.0.4 (Adamack & Gruber, 2014) and 'ggplot2' v. 3.3.5 (Wickham, 2016). This multivariate analysis decomposes the total variance of the genetic data into decreasing components, of which the first two (PC1 and PC2) were maintained. The graphical output provides a first look into the overall structure of the population and can point to outlying samples. The summary of the PCAs' output also provides basic statistics such as missing data, alleles per locus and population, allele frequencies, as well as observed and expected heterozygosity. Atypical samples detected in the PCAs (i.e., those that deviated from the main cluster of samples) were verified to identify the origin of their distinction; this may be due to a high amount of missing data, unique or rare allelic combinations, private alleles, or even spatial isolation.

Isolation by Distance (IBD) is a process of genetic differentiation increasing in a population along with the simultaneous increase of geographic distance (Frankham et al., 2010). Testing for the deviation from

the IBD is a common procedure in population genetic studies to identify potential disruptions in the movement of individuals across the landscape (Meirmans, 2015). In this case, the test was performed with the R packages 'fossil' v. 0.4.0. (Vavrek, 2011) and 'MASS' v. 7.3.53.1. (Venables et al., 2002). Here, a Mantel test (Sokal & Wartenberg, 1983) was applied using a matrix of genetic distances (pairwise distances between multivariate observations of allele frequencies) and the matrix of geographic distances, providing the basis to create a scatterplot that represents the pattern of genetic dissimilarity between individuals throughout the landscape (genetic distance on one axis and geographic distance in the other axis). The plot thus shows either a concentrated cloud of points following a linear increase (indicating IBD) or discontinued point clouds (which indicates a disruption of IBD and so the existence of patches) (Jombart, 2008). IBD is expected in cases where there are no significant barriers to dispersal, in which individuals who are further away from each other in space exhibit increasing genetic distance as well.

2.8 Spatial Genetic Analyses

2.8.1 Spatial Autocorrelation

A spatial structure autocorrelation analysis was conducted with GenAlEx to evaluate the relatedness degree between pairs of individuals, while considering their geographical distance (Peakall & Smouse, 2012). This analysis indicates if pairs of individuals are more related than expected at a given geographical distance in a random setting, thus pointing to the possibility of an underlying spatial genetic structure (Manel et al., 2003). It allows comparisons of the autocorrelation coefficient – r – that is generated for all pairs of individuals at each distance class. Although it does not provide a graphical pattern like in the case of the isolation by distance analysis, it provides the results on a finer geographic scale (Mbora & McPeek, 2015). For example, the amount of detail used in geographical distances can be selected by the researcher, thus allowing for a finer scale analysis of the genetic patterns across the landscape – as described below.

Firstly, the maximum distance between individuals was confirmed in the map of GRNP using QuantumGIS v. 3.16.10-Hannover (QGIS Development Team, 2002), to decide on the distance classes to be tested. The maximum distance between individuals was 69 km for RC and 64 km for BWC. To visualize the degree of relatedness between individuals belonging to the same social group, three spatial autocorrelation analyses were conducted – firstly with an even 2 km interval with an uneven start at the closest range (0, 0.2, 0.5, 1, 3, 5, 7, 9, (…) 61, 63, 64), then at a 3 km interval (0, 0.2, 0.5, 3, 6, 9, 12, 15, (…) 60, 63, 64), and at last, a 5 km interval (0, 0.2, 0.5, 5, 10, 15, 20, 25, (…) 55, 60, 64). In the case of the RC population, the intervals were equal except for the maximum distance at 69 km instead of 64km. All the analyses were performed with 999 permutations and 999 bootstraps. Significance of correlograms was determined at $p < 0.01$, following Banks & Peakall (2012). The genetic and geographic distance matrices were created, and the spatial autocorrelation analysis was performed – firstly for the complete populations and then for males and females separately. This separation can be helpful to understand if the genetic distance was being influenced by the dispersing sex or by the landscape itself.

2.8.2 Structure Tessellation

Due to the difficulties involved in inferring genetic clustering in a population, another spatial Bayesian clustering algorithm is also beneficial to add in a study of the structure of a population and the space it inhabits. TESS v. 2.3.1. (Chen et al., 2007) was used for this purpose, providing membership probabilities and geographical cluster assignments for every individual, without assuming predefined populations. This software provides a Hidden Markov Random Field as the no-admixture model and a Hidden Gaussian Random Field as the admixture model, to be applied on the spatial individual network (tessellation) that represents the assignments of individuals to clusters.

After computing geographical Euclidean distances, the first 10 independent runs without admixture would be performed for calibration of 2 to 11 clusters (K), using the predefined values (Spatial Interaction parameter = 0.6, Linear Trend = 1). Firstly, the sweeps and burn-in period selected were the same as the ones used in STRUCTURE, but that continuously crashed the program. After trial and error, the no-admixture model was applied with the same number of clusters and 10 independent runs for each K, but this time with 100,000 sweeps and a 10,000 burn-in period. The same parameters would be used for the Besag, York and Mollièe (BYM) model, which consists in modeling the spatial dependencies of admixture coefficients using a convolution Gaussian prior, and the Conditional Auto-Regressive (CAR) model, which is defined as a conditional auto-regressive Gaussian model that may represent the locally structured part of the variation (Durand et al., 2009a). Of these two models, the latter was selected as the one to be used for further interpretation, as its Deviance Information Criterion (DIC) was the lowest of the two (Durand et al., 2009b).

The number of potential clusters is expressed by the constant Kmax, which is usually larger than the presumably true number of clusters, K (Durand et al., 2009b). To select the Kmax and K value that best fits the data, the DIC values were plotted against Kmax to identify the value of K where it starts to plateau. For this purpose, the model without admixture was selected, as it seems that this is the best solution for detecting the maximal number of clusters (Basto et al., 2016; Safner et al., 2011). Since the value of Kmax may be greater than the true number of K, the interpretation of the resulting plots was complemented with an examination of the bar plots of estimated membership probabilities (Basto et al., 2016).

Afterwards, the runs were summarized and exported to CLUMPP v.1.1.2. (Jakobsson & Rosenberg, 2007), a software that corrects the individuals' percentages of assignment to each cluster across the different runs and calculates the mean of probabilities for each cluster. Thereafter, the summarized output was collected and displayed in a raster map of the park using the 'tess3r' v. 1.1.0. package in R (Caye et al., 2018). In other words, 'tess3r' illustrated the clustering of the populations on the protected area, which provides insights of the landscape features shaping the genetic patterns of these arboreal primates.

After a preliminary look at the outputs, another database was created for the BWC population without the previously detected highly related individuals. This dataset contained 18 samples and it was rerun with the same parameters as the full dataset, to confirm that the clustering was not a consequence of the information associated with highly related individuals.

2.9 Sex-biased Dispersal

The sex biased dispersal analysis involves, in this study, the calculation of mean corrected assignment indices (mAI_c) for males and females, with the purpose of identifying the dispersing sex. According to this method, the dispersing sex should be the one with a negative mAI_c (Goudet et al., 2002). This measure assumes that only post-dispersal individuals (adults) are included in the analysis. Since the fecal sampling strategy did not allow for the identification of the age-class of the individuals, it is very likely that there are both adult and juvenile individuals in the dataset, thus limiting the accuracy of the inference of the mAI_c estimates (Lawson, Handley & Perrin, 2007). This analysis was firstly performed with GenAlEx (Peakall & Smouse, 2012), removing the samples that had missing data – which is not accepted by GenAlEx in this case. When the removal of samples was not a viable option (resulting in a small sample number or complete removal of the samples from one of the sexes), the missing data was replaced with the most common allele of the database. Sex biased dispersal was calculated for BWC and RC complete datasets, then for forest blocks and for transects (putative social groups) that had enough samples and sex equilibrium. When comparing the results of tests without missing data and with replaced missing data, it was clear that the latter solution was skewing the results and thus, was not viable to solve the missing data constraint.

Due to the number of samples with missing data and consequent inadequacy of GenAlEx for this purpose, sex biased dispersal was further estimated with the 'hierfstat' v. 0.5.7 R package (Goudet, 2005). This test was performed on the complete BWC database, which was then subdivided into Gola South and Gola Central blocks and Transect 44 (the only one that had sufficient samples and sex equilibrium to perform the test with this dataset). The same was done to the RC database, being subdivided into Gola Central, Central North, Central with Central North, South, and Tiwai. Since this database is bigger than BWC's database, more transects were selected to perform the same tests (RT44, T37 and RT10, Figure 2.1). As all these individuals putatively belong to the same population (as inferred from the genetic structure analyses) and dispersal occurs between social groups, this subdivision into forest blocks and then transects (i.e., social groups) had the goal of increasing the chances of identifying the dispersal pattern of each species of colobus.

2.10 Demographic History

To search for genetic evidence of historical changes in the effective population size of the two colobines, a coalescent-based analysis of microsatellite variation was performed. More specifically, Markov chain Monte Carlo (MCMC) simulations were utilized to estimate the posterior probability of demographic parameters through simulations with the software MSVAR v. 1.3. (Beaumont, 1999; Storz & Beaumont, 2002). The hierarchical Bayesian method that is used as the basis for the simulations assumes a simple demographic model, which can involve a single point at which the effective population size changed and estimates the current (N_0) and ancestral (N_1) effective population sizes, as well as the time in generations (T) since that change occurred, assuming an exponential change in the population size. Here, the time is increasing into the past and the loci are assumed to be evolving according to a strict stepwise mutation model (SMM), with mutation rate per locus, μ. T was converted into years for the analyses and a generation time of 10 years was assumed for the two species (Gonedelé Bi et al., 2019; McGraw et al., 2020). Four independent runs were conducted for each of the three datasets that were used before: BWC with 15 loci and 25 individuals, BWC with 15 loci and 18 (unrelated) individuals and RC with 14 loci and 146 individuals. Each independent run had varied prior and hyperprior distributions, thus placing most of the prior support on different scenarios of constant population size (Run 1, $N_0=N_1$), bottleneck (Run 2, $N_0<N_1$), dramatic (Run 3, $N_0>>N_1$) and slight (Run 4, $N_0>N_1$) demographic expansion. The mutation rate was set to-3.5, supporting mutation rates of 10^{-4} to 10^{-3}, as specified in many demographic analyses (Storz & Beaumont, 2002). The standard variation was fixed to 1 (in a log10 scale) for all parameters. All runs were conducted with 300,000 thinned update steps, with a thinning interval of 30,000 – or in other words, $9x10^9$ steps. The first 10,000 (10%) iterations were discarded as burn in of results from each simulation to avoid bias in the parameters' estimation due to starting conditions. Checking for convergence between the different simulations (four for each dataset) to confirm their robustness was performed visually and via Brooks, Gelman and Rubin Convergence Diagnostic Test (Brooks & Gelman, 1998; Gelman & Rubin, 1992) implemented via R package 'boa' v. 1.1.8.2. (Smith, 2007). The referred test statistic is a multivariate potential scale reduction factor (MPSRF), which assesses the MCMC convergence by analyzing the difference between the several Markov chains. More specifically, the convergence is assessed by comparing the estimated variances between and within chains for a set of variables. As chains converge to a common distribution, the between-chain variability is expected to become smaller than the within-chain variability, expressed by an MPSRF close to 1. A 97.5% quantile greater than 1.20 is considered evidence of absence of convergence, which means values below 1.20 are considered to be a sign of convergence (Brooks & Gelman, 1998). After checking for convergence, the mean and median values of N_0, N_1, N_0/ N_1 and T were recorded and plotted, as well as their HPD 90% limits with the 'boa' R package.

3 Results

3.1 Quality control

Initially, the datasets for *C. polykomos* (further designated as BWC in this section) contained 54 samples with 15 loci and had an average QI of 72%, while the dataset for *P. b. badius* (further designated as RC in this section) had 198 samples with 15 loci and an average QI of 81%. I used the autosomal microsatellite loci D12s321, D2s136, D6s474, D10s611, D4s2408, D1s548, D2s442, D11s2002, D12s372, Fesps, D1s1665, D6s503, D6s1056, D10s676, D10s1432 and the sex-chromosome locus DeadBox for sexual identification. With these datasets, rates of allelic dropout and false allele errors (the most common errors) were calculated with GIMLET v. 1.3.3, with both species having low rates of the latter error. Overall, the results for errors in the BWC population averaged 11% for allelic dropout and 2% for false allele errors (Table 3.1). As for the RC population, it had even lower rates of allelic dropout (5%) and a smaller false allele rate (1%), before optimizations (Table 3.2). Afterwards, GEMINI v. 1.3.0 was used to assess the number of repeats that guaranteed maximum confidence in the genotypes. The value was set at four repeats, meaning that after four repetitions per sample, the quality would not be significantly improved. Therefore, four repeats were selected for each sample in both populations and all QIs were recalculated. We used three repeats for samples for which no more repeats were conducted. Furthermore, the samples and loci that had a QI lower than 0.31 and samples containing nine or more cases of no amplification were discarded from the databases. Additionally, after the last QI calculations, the locus D10s611 in the RC database presented a very low QI (0.33) and was consequently removed. At this point, there were 15 autosomal loci and 52 samples in the BWC database and 14 loci and 183 samples in the RC database. Subsequently, GenAlEx v. 6.5 was used to find repeated samples within each dataset calculating their probability of belonging to one individual. In the pairs of samples that were matched as probably equivalent, the ones with a lower QI and/or higher amount of missing were removed. After this process, the datasets had 42 BWC samples and 168 RC samples that we could confirm as probably unique.

Table 3.1 GIMLET v.1.3.3 error rates in the BWC population

Locus	Allelic Dropout	False allele
D13s321	0.127	0.071
D2s1326	0.098	0.021
D6s474	0.063	0.027
D10s611	0.035	0.006
D4s2408	0.049	0.010
D1s548	0.080	0.038
D2s442	0.008	0.015
D11s2002	0.019	0.031
D12s372	0.259	0.007
Fesps	0.061	0.017
D1s1665	0.166	0.022
D6s503	0.233	0.013
D6s1056	0.155	0.008
D10s676	0.147	0.027
D10s1432	0.111	0.014
Average	11%	2%

Table 3.2 GIMLET v. 1.3.3 Error rates in the RC population

Locus	Allelic Dropout	False allele
D13s321	0.029	0.024
D2s1326	0.009	0.012
D6s474	0.013	0.006
D10s611	0.105	0.007
D4s2408	0.027	0.015
D1s548	0.028	0
D2s442	0.158	0.03
D11s2002	0.013	0.007
D12s372	0.235	0.035
Fesps	0.015	0.022
D1s1665	0.005	0.005
D6s503	0.051	0.005
D6s1056	0.031	0.008
D10s676	0.024	0.018
D10s1432	0.013	0.006
Average	5%	1%

Subsequently, MICRO-CHECKER v. 2.2.3. (Van Oosterhout et al., 2004) was implemented to estimate scoring errors, allelic dropout, null alleles, and homozygote excess. In the dataset of RC, excess homozygotes were detected at loci D10s676, D6s503, and D2s442. A scoring error was detected in locus D6s503, and null alleles were present in loci D10s676, D6s503 and D2s442 (Table 3.3). In the dataset of BWC, the program found excess homozygotes at loci D10s676, D6s503, D1s1665, D1s548, D4s2408, and D2s136. Scoring errors were also found at loci D10s676 and D1s1665. Lastly, null alleles were observed at loci D10s676, D6s503, D1s1665, D1s548, D4s2408 and D2s136 (Table 3.3). Next, Genepop on the Web v. 4.7.5. was used to check for deviations from HWE and presence of LD. After applying the Bonferroni correction (p value = 0.003 for BWC and p value = 0.0036 for RC), the loci D2s442, D12s372 and D6s503 in the RC population and loci D1s1665, D6s503 and D10s676 of the BWC population were observed to deviate from HWE (Table 3.3.). These results are concordant with the output from MICRO-CHECKER, as all loci (except locus D12s372 in RC) also presented excess homozygotes, presence of null alleles and even some scoring errors. LD was detected only in the RC population, specifically in loci D4s2408 with Fesps, D1s1665 with D11s2002 and D10s676 with Fesps, with no significant results for this analysis in the BWC population (Table 3.3.).

Table 3.3 Summary of loci with error rates, significant deviations from Hardy-Weinberg Equilibrium, and involved in pairs where Linkage Disequilibrium was observed, after Bonferroni corrections in the *P. b. badius* (RC) (p value = 0.0036) and *C. polykomos* (BWC) (p value = 0.003) populations. Error rates resulted from MICRO-CHECKER v. 2.2.3. and HWE and LD from Genepop on the Web v. 4.7.5.

MICRO-CHECKER Errors	Hardy-Weinberg	Linkage Disequilibrium
P. b. badius (RC)		
D10s676	D2s442	D4s2408 - Fesps
D6s503	D12s372	D11s2002 - D1s1665
Ds442	D6s503	Fesps - D10s676
C. polykomos (BWC)		
D10s676	D1s1665	–
D6s503	D6s503	–
D1s1665	D10s676	–
D1s548	–	–
D4s2408	–	–
D2s136	–	–

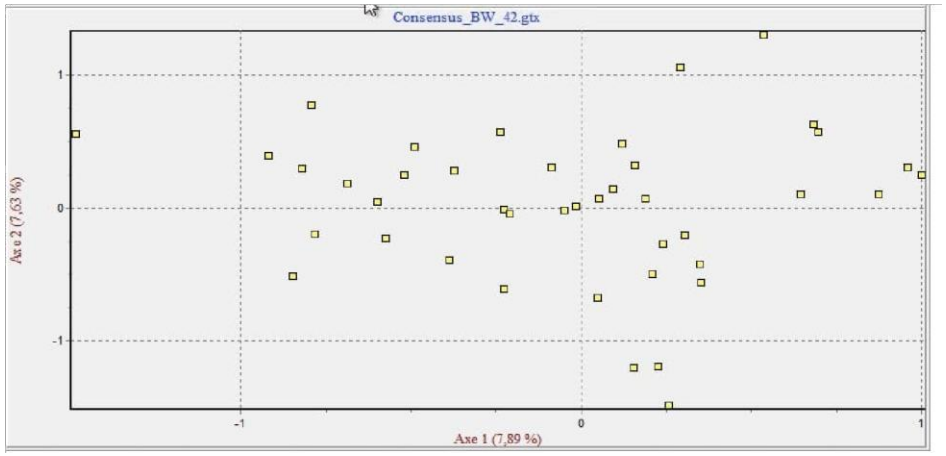


Figure 3.1 Factorial Correspondence Analysis of 42 samples of BWC. Produced in GENETIX v. 4.05.

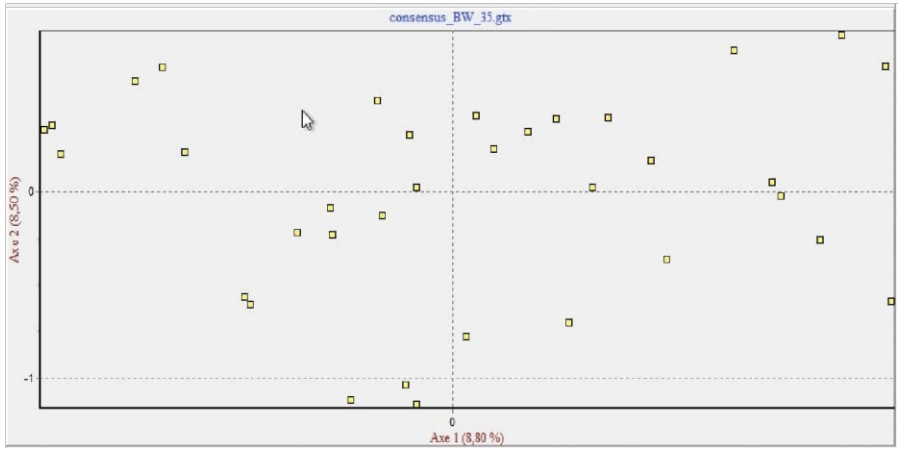


Figure 3.2 Factorial Correspondence Analysis of 35 samples of BWC. Produced in GENETIX v. 4.05.

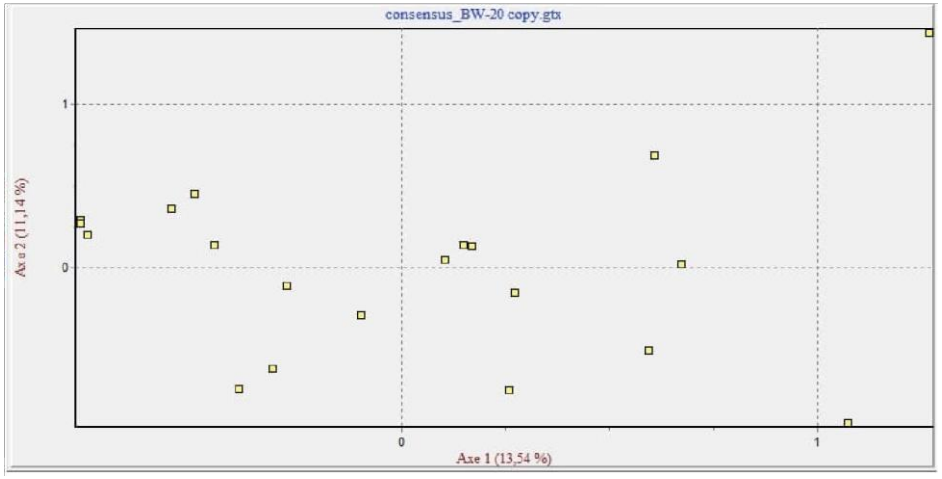


Figure 3.3 Factorial Correspondence Analysis of 20 samples of BWC. Produced in GENETIX v. 4.05.

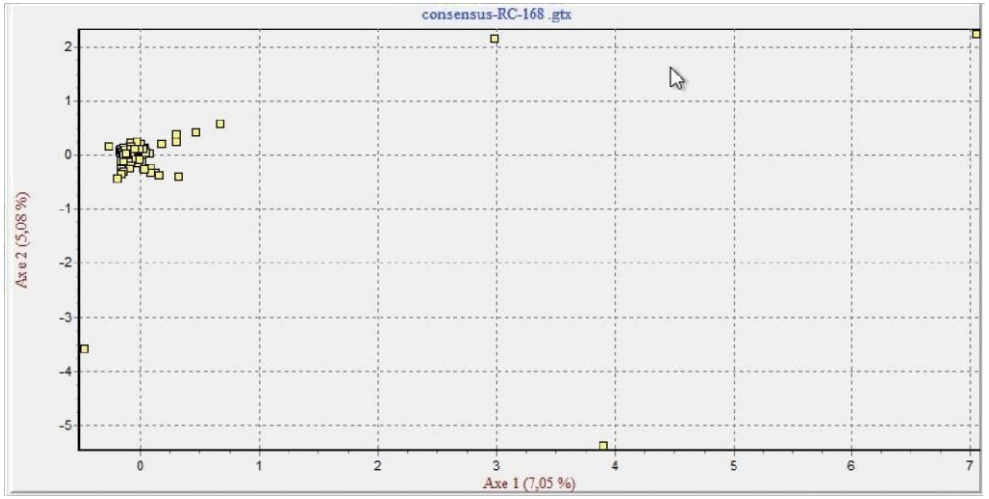


Figure 3.4 Factorial Correspondence Analysis of 168 samples of RC. Produced in GENETIX v. 4.05.

Figure 3.5 Factorial Correspondence Analysis of 162 samples of RC. Produced in GENETIX v. 4.05.

Figure 3.6 Factorial Correspondence Analysis of 149 samples of RC. Produced in GENETIX v. 4.05.

After recalculations of HWE and LD and optimizations of data quality, the final dataset of the BWC population contained 25 samples (15 females and 10 males) with 15 microsatellite loci, with an average QI of 85% throughout all loci (QI per locus in Table 3.4). Additionally, the final dataset of the RC population contained 146 samples (96 males and 52 females) and 14 microsatellite loci, with an average QI of 90% (QI per locus in Table 3.5). The lowest QI in a BWC sample was 66% and all samples had < 5 genotypes with missing data. The lowest QI of a RC sample was 71% and, since this database had more samples, the limit of missing data was < 3 genotypes. Both QI values per locus and per sample are higher than the conventional limits (0.625) for QI values (Miquel et al., 2006), which is a good indicator of the quality of the genetic data used for this study.

Now, a decision had to be made about the problematic loci (presenting errors in MICRO-CHECKER, deviations from HWE and presence of LD) – as these could bias following analyses. Looking at the summary of loci with these errors (Table 3.3), it was decided that the ones appearing in all the different tests would be selected as the most problematic. In the BWC population, the loci D10s676, D6s503 and D1s1665 appeared more involved in errors in the MICRO-CHECKER tests and were all deviating significantly from HWE. In the RC population, the loci D6s503 and Ds442 were the ones with most errors in MICRO-CHECKER and with significant deviations from HWE. Considering the existence of these three problematic loci in the BWC population and those two in the RC population, an additional dataset was created for each species without these loci. Thus, subsequent analyses were conducted using two datasets per population (25 samples of BWC with 15 and 12 loci, 146 samples of RC with 14 and 12 loci) to ensure that results were not being biased by the errors that these loci contained. Their comparison would help in the decision on which results should be discussed.

Table 3.4 Calculated quality index (QI) per locus and average QI for the BWC population.

Locus	QI %
D13s321	0.78666667
D2s136	0.96
D6s474	0.93333333
D10s611	0.96
D4s2408	0.86666667
D1s548	0.62666667
D2s442	0.78666667
D11s2002	0.78666667
D12s372	0.72
Fesps	0.93333333
D1s1665	0.96
D6s503	0.82666667
D6s1056	0.86666667
D10s676	0.78666667
D10s1432	0.97333333
Average	**0.85155556**

Table 3.5 Calculated quality index (QI) per locus and average QI for the RC population.

Locus	QI %
D13s321	092351598
D2s1326	0.98858447
D6s474	0.94349315
D4s2408	0.9760274
D1s548	0.9326484
D2s442	0.66152968
D11s2002	0.96232877
D12s372	0.80022831
Fesps	0.90125571
D1s1665	0.96347032
D6s503	0.8413242
D6s1056	0.97488584
D10s676	0.88013699
D10s1432	0.97773973
Average	**0.9090835**

3.2 Genetic Diversity

Genetic diversity parameters were gathered from literature and calculated as previously described for the two species. The data contained unidentified pre- and post-dispersal individuals and were summarized by locus (Table 3.6 and Table 3.7). The mean number of alleles (N_a) was higher in RC (8.857) than in BWC (5.267), ranging from 5 to 15 alleles in the former and from 3 to 7 in the latter. This observation is the same in the case of the number of effective alleles (N_e), with a mean of 4.092 for RC and 3.172 for BWC.

Table 3.6 Genetic diversity indices for the microsatellite loci of the *C. polykomos* (BWC) population from GenAlEx v. 6.5 extension of Excel and 'hierfstat' and 'adegenet' R packages; N = Number of alleles, N_a = Number of different alleles, N_e = Number of Effective alleles, H_o = Observed Heterozygosity, H_e = Expected Heterozygosity, A_r =Allelic Richness, F_{is} = Inbreeding Coefficient. Hardy-Weinberg Equilibrium (HWE) and respective Bonferroni correction, with significant deviations from HWE considering Bonferroni correction in bold, calculated in 'Genepop on the Web' v. 4.7.5. SE = Standard Error.

Locus	N	N_a	N_e	H_o	H_e	A_r	F_{is}	HWE (Bonferroni =0.003)
D13s321	23	6	4.180287	0.61	0.76	5.615166	0.221	0.088
D2s136	25	7	3.180662	0.48	0.69	6.241709	0.318	0.005
D6s474	24	5	3.755177	0.88	0.73	4.926694	0.172	0.499
D10s611	25	3	1.862891	0.48	0.46	2.996756	0.016	1.00
D4s2408	22	6	3.239318	0.5	0.69	5.833349	0.298	0.025
D1s548	18	7	5.6866	0.67	0.82	6.747515	0.218	0.091
D2s442	21	5	2.890132	0.67	0.65	4.574959	0.005	0.408
D11s2002	20	7	5,00	0.8	0.8	6.612517	0.026	0.828
D12s372	20	5	1.830664	0.35	0.45	4.79873	0.253	0.322
Fesps	24	4	2.613382	0.5	0.62	3.611117	0.21	0.218
D1s1665	25	6	3.369272	0.44	0.7	5.122548	0.392	**0.002**
D6s503	22	4	1.877539	0.14	0.47	3.87192	0.72	**0.00**
D6s1056	23	5	1.897483	0.43	0.47	4.369172	0.104	0.103
D10s676	21	4	2.990091	0.14	0.67	3.999196	0.795	**0.00**
D10s1432	25	5	3.213368	0.76	0.69	4.820464	0.083	0.285
Average	**22.53**	**5.267**	**3.172**	**0.523**	**0.645**	**4.943**	**0.219**	**0.258**
SE	**0.568**	**0.316**	**0.295**	**0.056**	**0.032**	**0.285**	**0.071**	**0.001**

Table 3.7 Genetic diversity indices for the microsatellite loci of the *P. b. badius* (RC) population from GenAlEx v. 6.5 extension of Excel and 'hierfstat' and 'adegenet' R packages; N = Number of alleles, N_a = Number of different alleles, N_e = Number of Effective alleles, H_o = Observed Heterozygosity, H_e = Expected Heterozygosity, A_r =Allelic Richness, F_{is} = Inbreeding Coefficient. Hardy-Weinberg Equilibrium (HWE) and respective Bonferroni correction, with significant deviations from HWE considering Bonferroni correction in bold, calculated in 'Genepop on the Web' v. 4.7.5. SE = Standard Error.

Locus	N	N_a	N_e	H_o	H_e	A_r	F_{is}	HWE (Bonferroni =0.0036)
D13s321	141	8	3.07	0.62	0.67	7.42	0.09	0.4
D2s1326	146	14	5.18	0.83	0.81	12.45	-0.02	0.89
D6s474	142	15	5.89	0.81	0.83	13.54	0.03	0.02
D4s2408	146	9	5.53	0.82	0.82	8.31	0	0.88
D1s548	142	9	4.42	0.81	0.77	8.25	-0.04	0.4
D2s442	110	8	2.57	0.4	0.61	7.72	0.35	**0**
D11s2002	143	8	4.33	0.71	0.77	7.6	0.08	0.13
D12s372	137	5	1.66	0.5	0.4	4.51	-0.26	**0**
Fesps	135	8	5	0.8	0.8	7.61	0	0.58
D1s1665	141	5	3.17	0.63	0.68	5	0.08	0.23
D6s503	128	9	2.29	0.12	0.56	8.77	0.79	**0**
D6s1056	145	7	3.01	0.76	0.67	6.61	-0.13	0.33
D10s676	136	9	6.51	0.76	0.85	8.65	0.11	0.07
D10s1432	145	10	4.66	0.78	0.79	9.33	0.01	0.11
Average	**138.36**	**8.857**	**4.092**	**0.668**	**0.716**	**8.270**	**0.077**	**0.29**
SE	**2.565**	**0.748**	**0.393**	**0.054**	**0.033**	**0.647**	**0.066**	**0.001**

Finally, the same trend of higher values in the RC population than in the BWC one is seen with observed heterozygosity (H_o) (0.668 and 0.523 respectively), expected heterozygosity (H_e) (0.716 and 0.645 respectively), and allelic richness (A_r) (8.270 and 4.943 respectively). As for the inbreeding coefficient (F_{is}), it presented itself significant in the BWC (F_{is} = 0.219, lower limit of Confidence Interval = 0.0892), but not in the RC (F_{is} = 0.077 lower limit of Confidence Interval = -0.0129). Lastly, the loci D6s474, D2s442, D12s372 and D6s503 (Bonferroni *p* value = 0.0036) deviated from HWE in the RC and loci D1s1665, D6s503 and D10s676 (Bonferroni *p* value = 0.003) deviated from HWE in the BWC. These results suggest that the RC population is overall more genetically diverse and less inbred than the BWC population.

3.3 Relatedness

Relatedness patterns are connected to several analyses used here subsequently, the presence of which can bias results. A preliminary run on the STRUCTURE v. 2.3.4. program was performed to guide the relatedness analysis (for details, see 3.4 Populations' Structure section below). Since no clear structure was detected in the RC's case, it was decided that relatedness wouldn't be a necessary analysis in that case. As for the BWC dataset, the preliminary STRUCTURE run showed clear substructuring in the population, which determined the need to check for relatedness in this case. For this purpose, the software Kingroup v. 2 was used, revealing several pairs of related individuals (*p* value = <0.05) in both the full siblings VS unrelated and parent/offspring VS unrelated tests. To have these samples in consideration when performing additional analyses on a dataset without highly related individuals, we would have to create a dataset with very few individuals. For this reason, a rerun was performed, this time considering high relatedness (*p* value <0.001). Some related pairs were again identified (but not as

many) and separated for another STRUCTURE analysis where it was possible to confirm that the substructure in the population was in fact due to their presence. Consequently, they were separated for another dataset of 18 samples to perform further analyses of genetic structure, spatial genetic structure and demographic history in the BWC population (STRUCTURE, TESS and MSVAR programs respectively, for details see following sections) with and without these highly related individuals.

3.4 Populations' Structure

In order to detect possible substructuring within the two populations, the software STRUCTURE v. 2.3.4. was used to calculate the probability of the individuals belonging to different genetic groups. Visual inspection of STRUCTURE outputs (Figure 3.7 & Figure 3.11 (K=2); Figure 3.8 & Figure 3.12 (K=3)) and Structure Harvester v. 0.6.94 through Evanno's ΔK method (Figure 3.13 & Figure 3.17) and inspection of L (K) (Figure 3.14 & Figure 3.18) (Evanno et al., 2005), suggest that the most probable number of clusters for both species was K=2. We can observe this on the graphical results, as there are individuals seeming to belong more to one cluster than another (green or red). To investigate whether the inferred genetic clustering was due to population differentiation or induced by the social group structure, I removed the seven highly related individuals from the BWC population (Rodr.guez-Ramilo & Wang, 2012), and conducted another STRUCTURE analysis (Figure 3.9 & Figure 3.10), confirming the latter expectation. Once the highly related individuals were removed from the BWC database, every individual showed an equal probability of belonging to each of the clusters and therefore an absence of structure (K=1) (Table 3.9, Figure 3.9 & Figure 3.10). In other words, each individual seemed to belong 50% to each cluster (green and red) when K=2 and 33% to each cluster (red, green and blue) when K=3. This is a pattern that is true when K=1, even though the Evanno's ΔK and of L (K) gave K=3 as the most probable option (Figure 3.15 & Figure 3.16). To corroborate once again all the results and determine if there was substructuring within the two clusters in each population, a calculation of Posterior Probability was performed (Table 3.8 for BWC, Table 3.10 for RC), which showed once again K=2 as the most probable option for both species (probability of 1.00 for BWC and probability of 0.99680 for RC). Therefore, in the case of BWC, the substructure was explained by the presence of a group of highly related individuals, confirmed by the absence of any substructure without these high- relatedness samples (K=1, Table 3.9). For this reason, the following structure analysis results will not include this dataset without the highly related individuals for discussion. Furthermore, the results for RC seem to indicate the existence of two genetic clusters in the population, but with most of the individuals being an of admixed ancestry and with no clear genetic differentiation between groups of individuals.

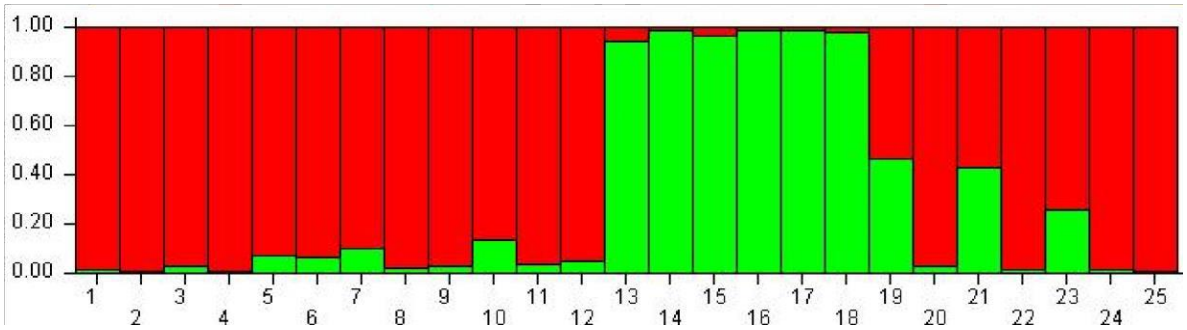


Figure 3.7 Genetic population structuring of the BWC population with 25 individuals. Each line represents a single individual, while the different colors represent genetic clusters. Here, the probability of K = 2 is simulated. Produced with STRUCTURE v. 2.3.4.

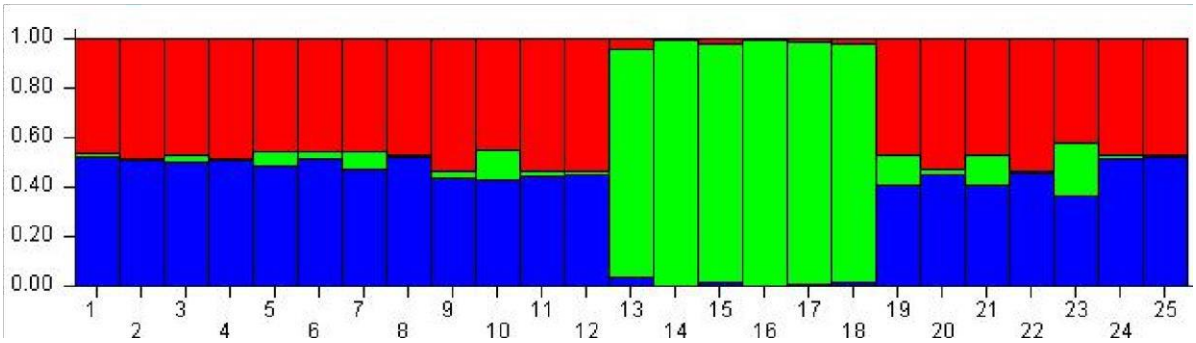


Figure 3.8 Genetic population structuring of the BWC population with 25 individuals. Each line represents a single individual, while the different colors represent genetic clusters. Here, probability of K = 3 is simulated. Produced with STRUCTURE v. 2.3.4.

Figure 3.9 Genetic population structuring of the BWC population with 18 individuals. Each line represents a single individual, while the different colors represent genetic clusters. Here, probability of K = 2 is simulated. Produced with STRUCTURE v. 2.3.4.

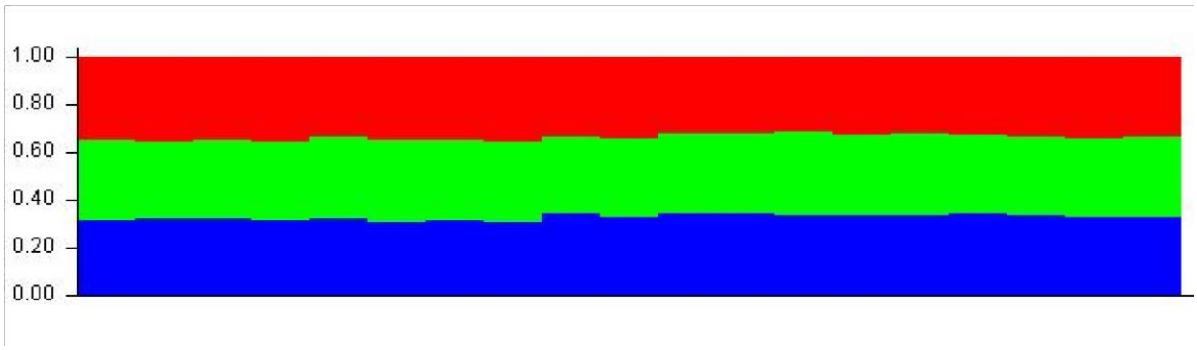


Figure 3.10 Genetic population structuring of the BWC population with 18 individuals. Each line represents a single individual, while the different colors represent genetic clusters. Here, probability of K = 3 is simulated. Produced with STRUCTURE v. 2.3.4.

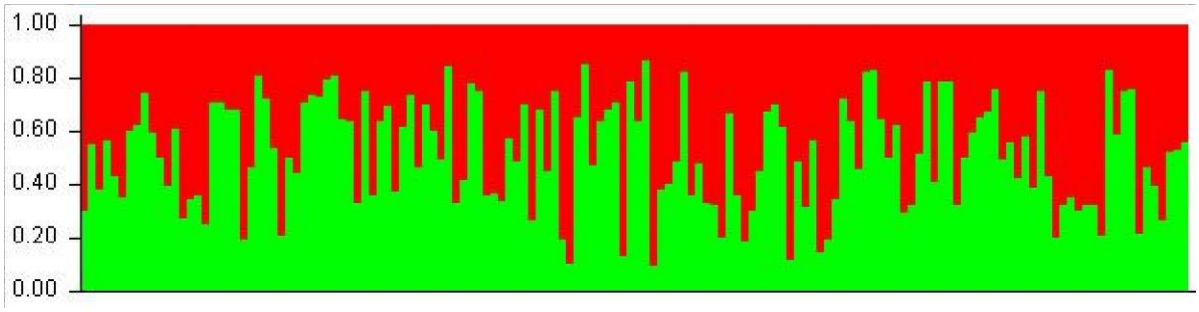


Figure 3.11 Genetic population structuring of the RC population with 146 individuals. Each line represents a single individual, while the different colors represent genetic clusters. Here, probability of K = 2 is simulated. Produced with STRUCTURE v. 2.3.4.

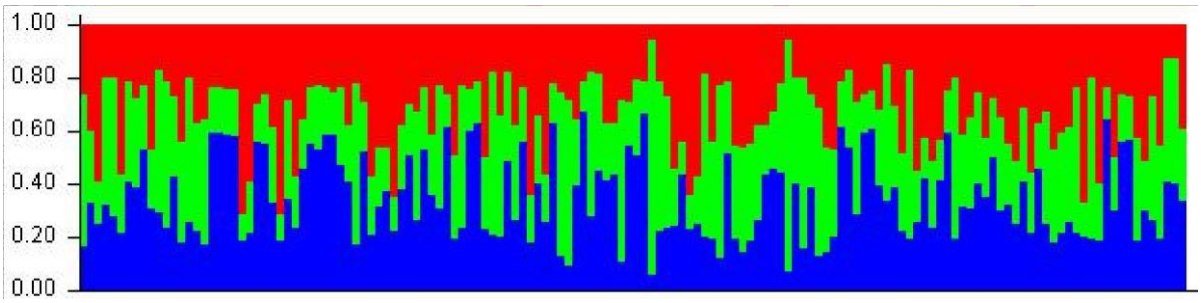


Figure 3.12 Genetic population structuring of the RC population with 146 individuals. Each line represents a single individual, while the different colors represent genetic clusters. Here, probability of K = 3 is simulated. Produced with STRUCTURE v. 2.3.4.

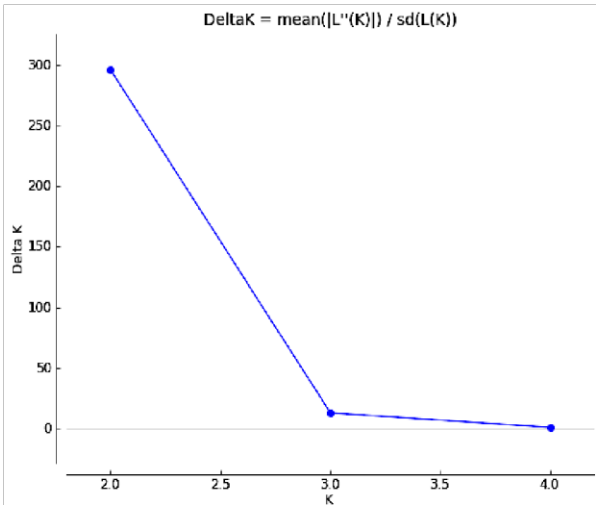


Figure 3.13 Graphical representation of best fitting K by Evanno's Δ K method for the BWC population with 25 individuals. Produced with Structure Harvester v. 0.6.94.

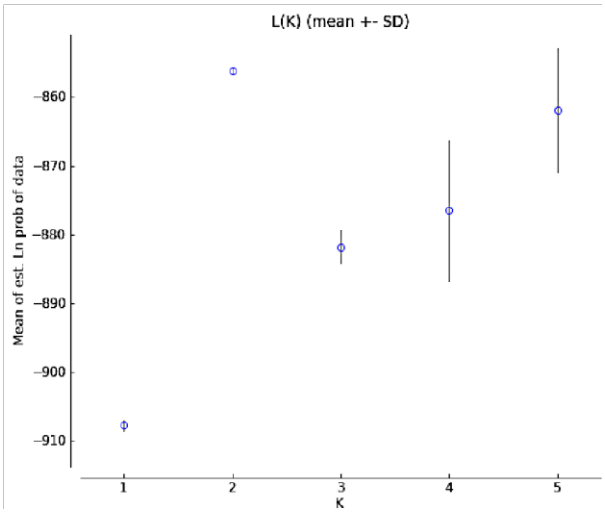


Figure 3.14 Graphical representation of L (K) analysis for the BWC population with 25 individuals. Produced with Structure Harvester v. 0.6.94.

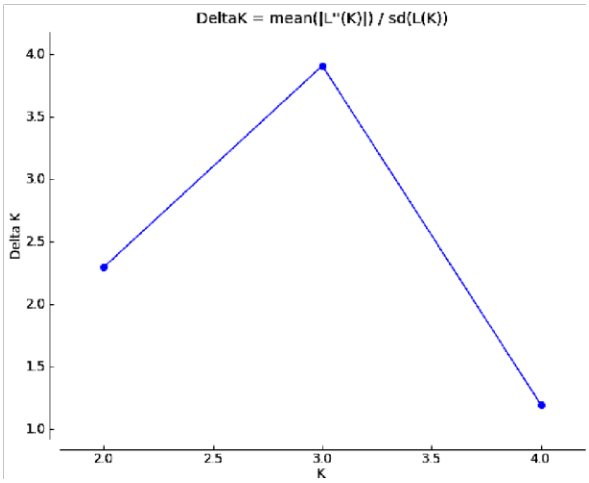


Figure 3.15 Graphical representation of best fitting K by Evanno's Δ K method for the BWC population with 18 individuals. Produced with Structure Harvester v. 0.6.94.

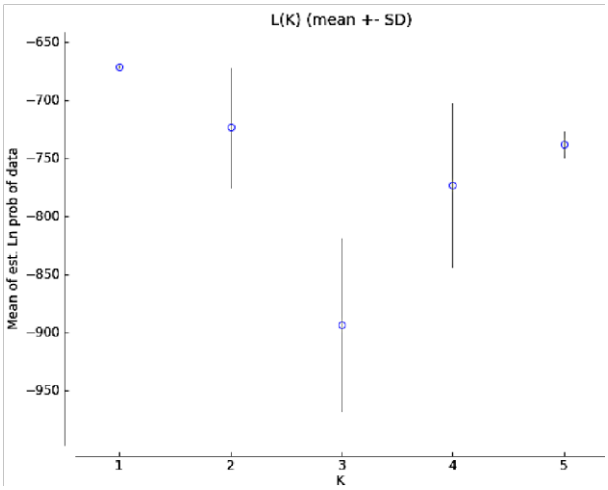


Figure 3.16 Graphical representation of L (K) analysis for the BWC population with 18 individuals. Produced with Structure Harvester v. 0.6.94.

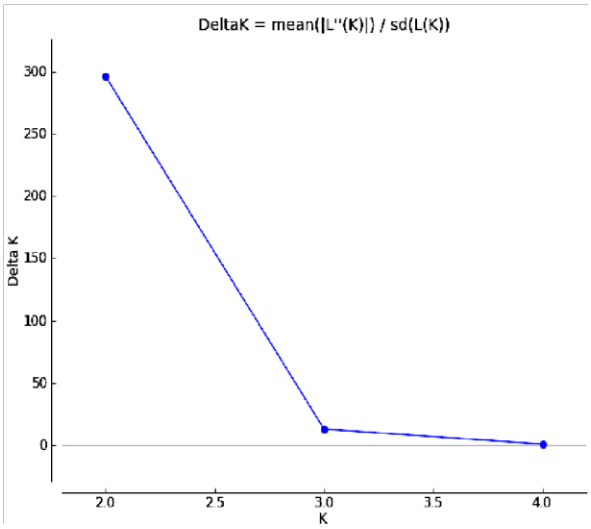


Figure 3.17 Graphical representation of best fitting K by Evanno's ΔK method for the RC population. Produced with Structure Harvester v. 0.6.94.

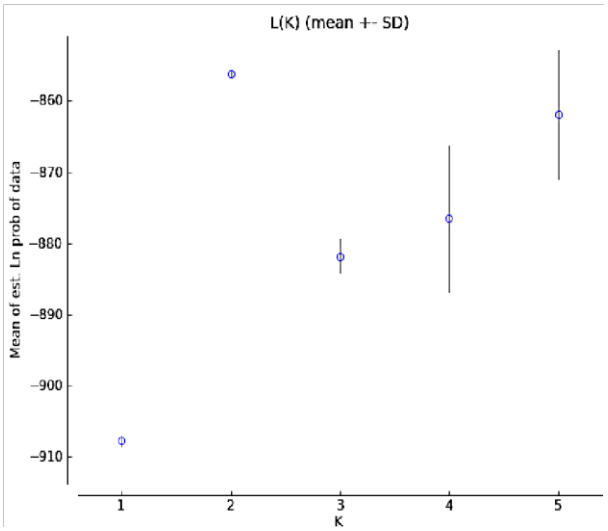


Figure 3.18 Graphical representation of L (K) analysis for the BWC population with 18 individuals. Produced with Structure Harvester v. 0.6.94.

Table 3.8 Calculation of Posterior Probability for the BWC (15 loci, 25 individuals) population, with most probable K in bold.

BWC – 15-25	**K**	1	**2**	3	4	5
K	Ln(P)	-907.70	-856.16	-881.78	-876.48	-861.90
1	-907.70	-1.00	-52.54	-26.92	-32.22	-46.80
2	-856.16	50.54	-1.00	24.62	19.32	4.74
3	-881.78	24.92	-26.62	-1.00	-6.30	-20.88
4	-876.48	30.22	-21.32	4.30	-1.00	-15.58
5	-861.90	44.80	-6.74	18.88	13.58	-1.00
Probability	–	0.00	**1.00**	0.00	0.00	0.00

Table 3.9 Calculation of Posterior Probability for the BWC (15 loci, 18 individuals) population, with most probable K in bold.

BWC – 15-18	**K**	**1**	2	3	4	5
K	Ln(P)	-671.68	-723.80	-893.66	-773.64	-738.02
1	-671.68	-1.00	51.12	220.98	100.96	65.34
2	-723.80	-53.12	-1.00	168.86	48.84	13.22
3	-893.66	-222.98	-170.86	-1.00	-121.02	-156.64
4	-773.64	-102.96	-50.84	119.02	-1.00	-36.62
5	-738.02	-67.34	-15.22	154.64	34.62	-1.00
Probability	–	**1.00**	0.00000	0.00	0.00	0.00

Table 3.10 Calculation of Posterior Probability for the RC (14 loci, 146 individuals) population, with most probable K in bold.

RC – 14 - 146	**K**	1	**2**	3	4	5
K	Ln(P)	-907.7	-856.16	-881.78	-876.48	-861.9
1	-907.7	-1.00	-52.54	-26.92	-32.22	-46.80
2	-856.16	50.54	-1.00	24.62	19.32	4.74
3	-881.78	24.92	-26.62	-1.00	-6.29	-20.88
4	-876.48	30.22	-21.32	4.29	-1.00	-15.58
5	-861.9	44.80	-6.740	18.88	13.58	-1.00
Probability	–	0.00	**0.99680**	0.00	0.00	0.00320

The PCAs were performed in RStudio v. 1.4.1106 using the allele frequencies of each population, providing a graphic summary of the genetic diversity of the BWC and RC populations. In the first species, two outlying individuals were detected (Figure 3.19); in total, they contained five occurrences of missing data, nine unique allelic combinations, and two private alleles. At the same time, the outlying individuals – UN112507 and UN112506 – were the only samples present in the Transect 10, which was situated between two other transects (Figure 2.1). Beside this detail, no substructure was apparent in the PCA of the BWC population, as all the samples were clearly connected to one cluster and almost all of them were contained by its inertia ellipse. As for RC, there were also two outlying individuals: RC050624 and RC042217 (Figure 3.20). Their genotypes, geographic position and electropherograms were subjected to careful inspection, but no other reasons were found that could explain their differentiation from the rest of the population. The PCA of this population shows a homogeneous distribution of the individuals inside the inertia ellipse, all connected to one cluster – suggesting overall substructure absence.

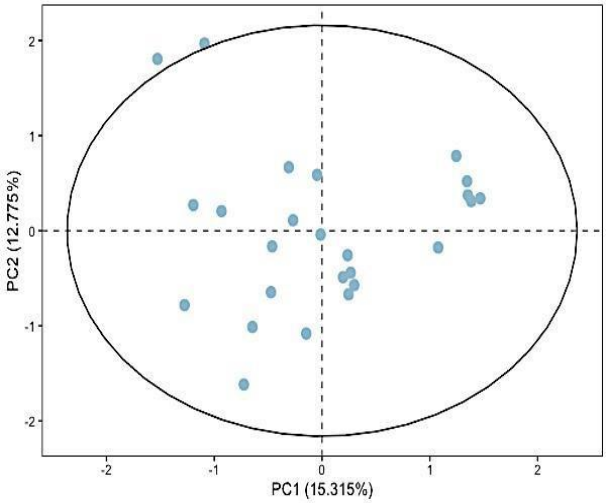


Figure 3.19 Principal Component Analysis of the BWC population with 25 individuals. Produced in RStudio v. 1.4.1106.

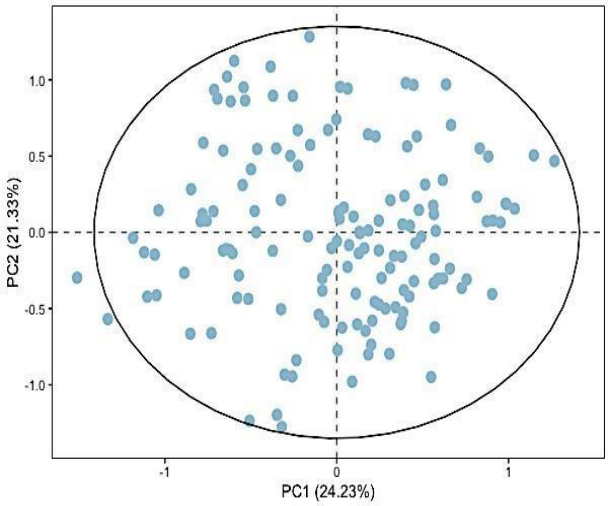


Figure 3.20 Principal Component Analysis of RC population. Produced in RStudio v. 1.4.1106.

Isolation by Distance (IBD) is the process of genetic differentiation increasing along with the incremental geographic distance in a population. To test for the existence of the IBD pattern, a Mantel test was performed, and the results were summarized and plotted in histograms (Figure 3.21 for the BWC and Figure 3.22 for the RC). For the BWC, isolation by distance was clearly significant (*p* value < 0.001) – contrasting with the results in the RC case (*p* value = 0.4687). To provide more detail to the analysis, a scatterplot was created to clearly observe the distribution patterns of the population. The graph is supposed to represent a linear cloud of points in the presence of IBD, while in the absence of it, there will be more than one concentrated point cloud with a clear separation. Once again, the BWC population (Figure 3.23) shows a pattern of IBD without discontinuities, while the RC population (Figure 3.24) shows a clear division and so, a disruption of the IBD pattern. This means that, for the BWC population, Euclidean geographical distance is shaping the distribution of the genetic diversity across space, while for the RC population other factors may explain their spatial genetic structure.

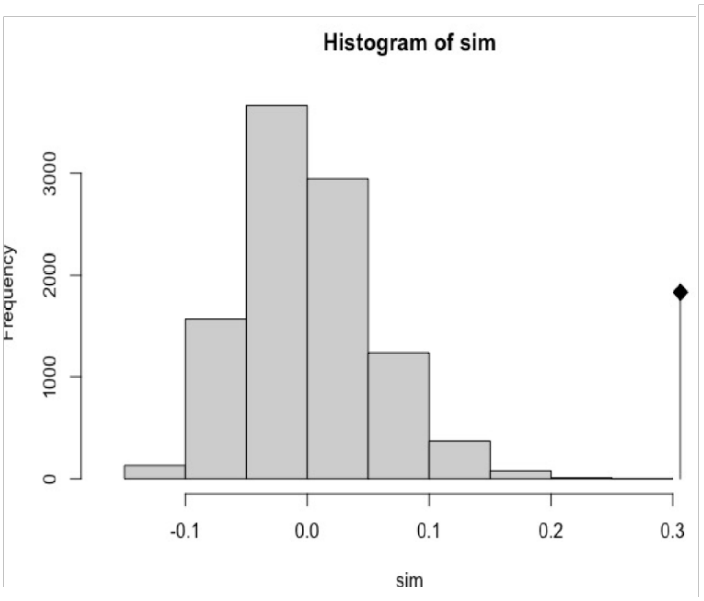


Figure 3.21 Mantel test for IBD of the BWC population. The original value is represented by the dot, while histograms represent permuted values. Here, the former is located out of the reference distribution, indicating IBD (*p* value = <0.001). Produced in RStudio v. 1.4.1106.

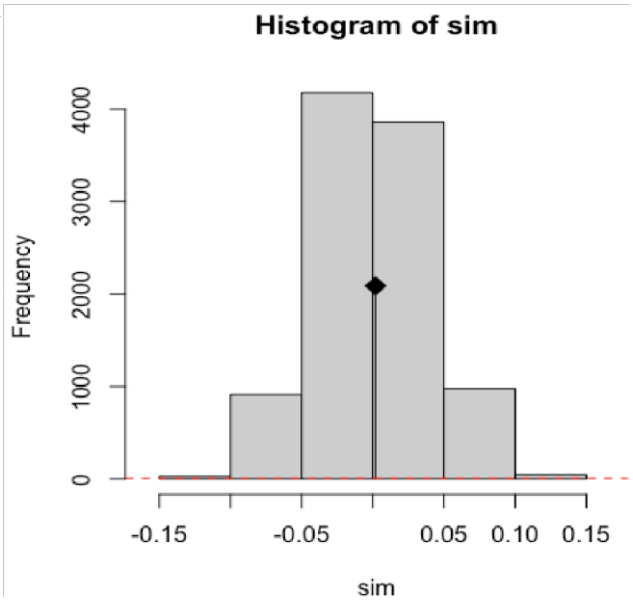


Figure 3.22 Mantel test of IBD of the RC population. Here, the original value of the correlation between the distance matrices is located out located out of the reference distribution, indicating disruption of the IBD pattern (p value = 0.4687). Produced in RStudio v. 1.4.1106.

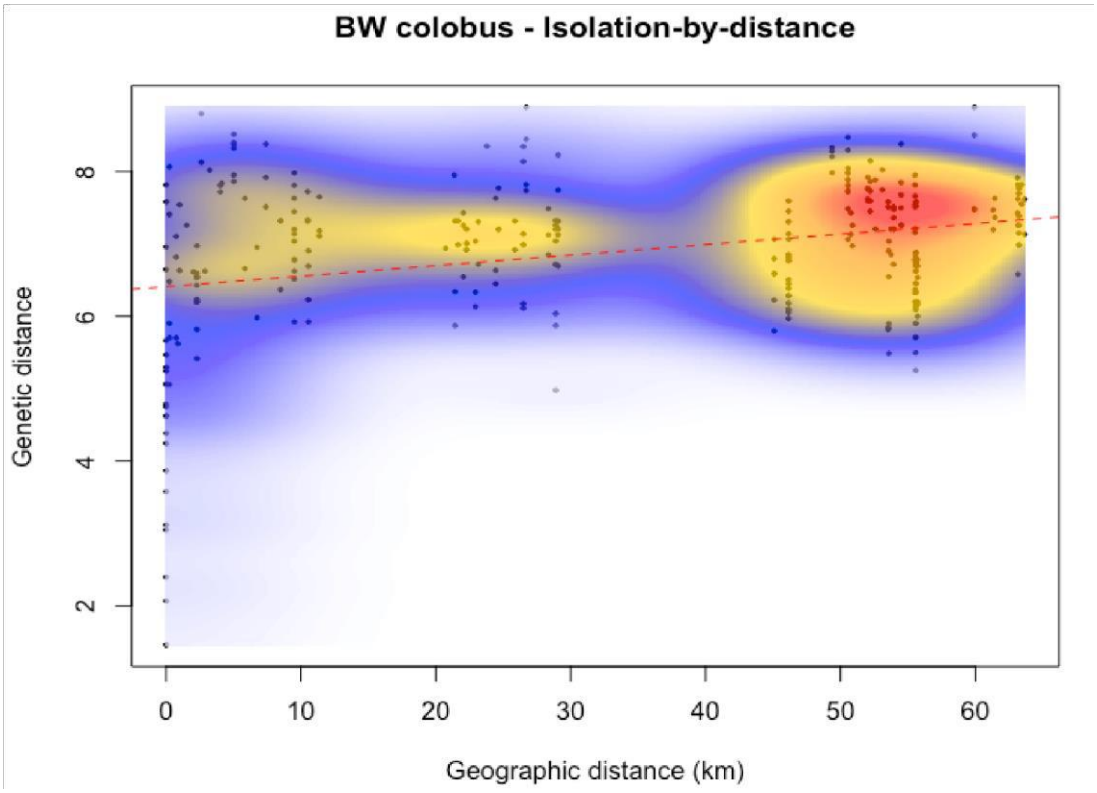


Figure 3.23 Scatterplot representing the IBD pattern in the BWC population, in the absence of discontinuities in the point cloud (r = 0.3062773). Produced with RStudio v. 1.4.1106.

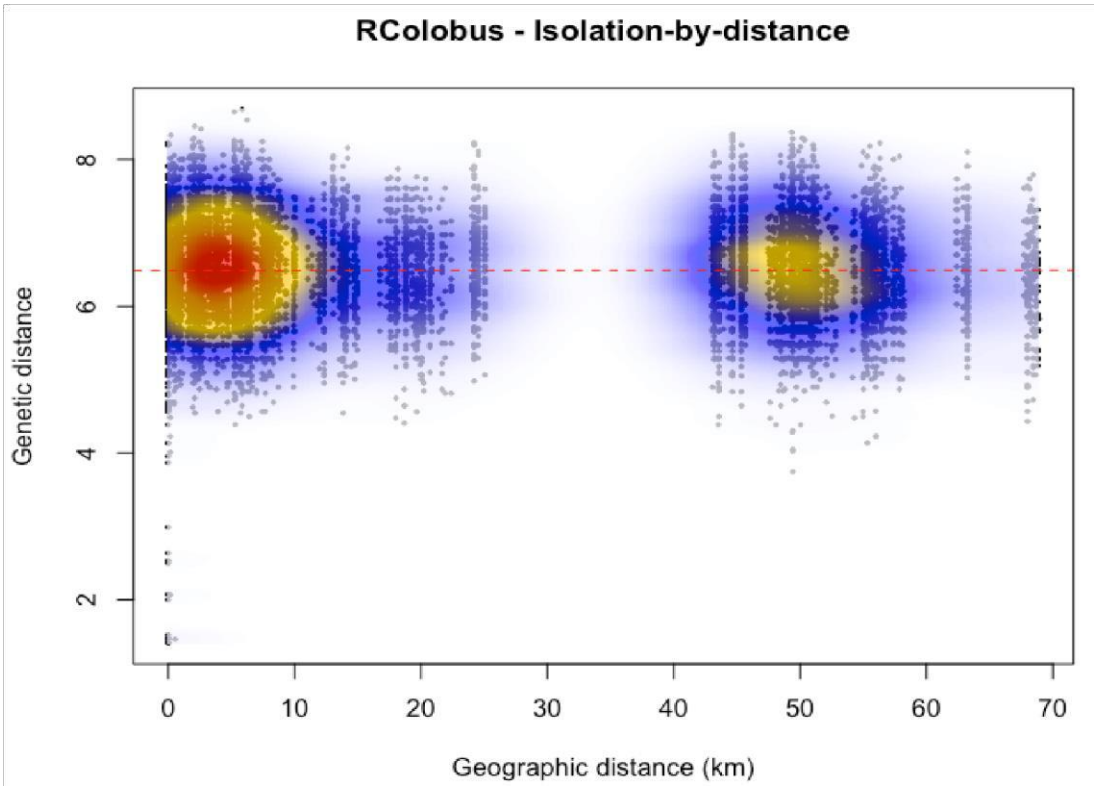


Figure 3.24 Scatterplot representing the disruption of the IBD pattern in the RC population, in the presence of discontinuities that created two clouds of points (r = 0.001909559). Produced with RStudio v. 1.4.1106.

3.5 Spatial Genetic Analyses

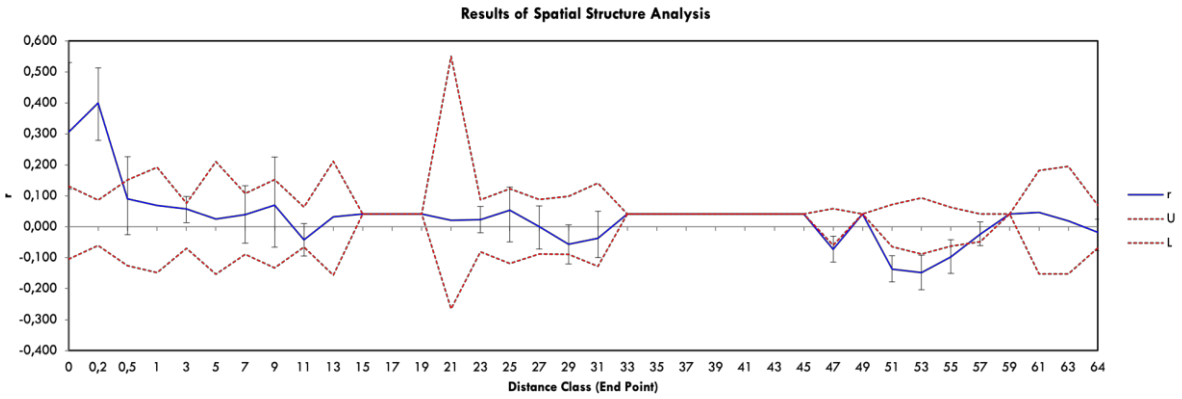
3.5.1 Spatial Autocorrelation

To evaluate the relatedness degree between pairs of individuals while considering simultaneously their geographical distance, the Spatial Autocorrelation analysis was conducted. At first, the spatial autocorrelation test was performed on the whole BWC population with a distance interval of 2 km (Figure 3.25 a)), 3 km (p value = 0.006) (Figure 3.25 b)) and 5 km (p value = 0.003) (Figure 3.25 c)). The only non-significant (p value = 0.038) correlogram was the former, but all the graphs had a higher autocorrelation coefficient (r) at the 0.2 km (r = 0.399) and a lower r from ~46 km to 57 km (r = -0.137 at 3 km interval, r = -0.120 at 5 km interval), where it extended outside the boundaries of the 95% confidence intervals. Hence, it is possible to conclude that the highest relatedness is found at the shortest distances and therefore at the social group level, which is expected from animals that gather to live in groups. The results also suggest more dissimilarity between dyads than expected at random at the furthest distances, far from the original social group of the individuals. Also, all the analyses show a gap at about 33 to 45 km, which is the distance between the Gola South and Gola Central blocks.

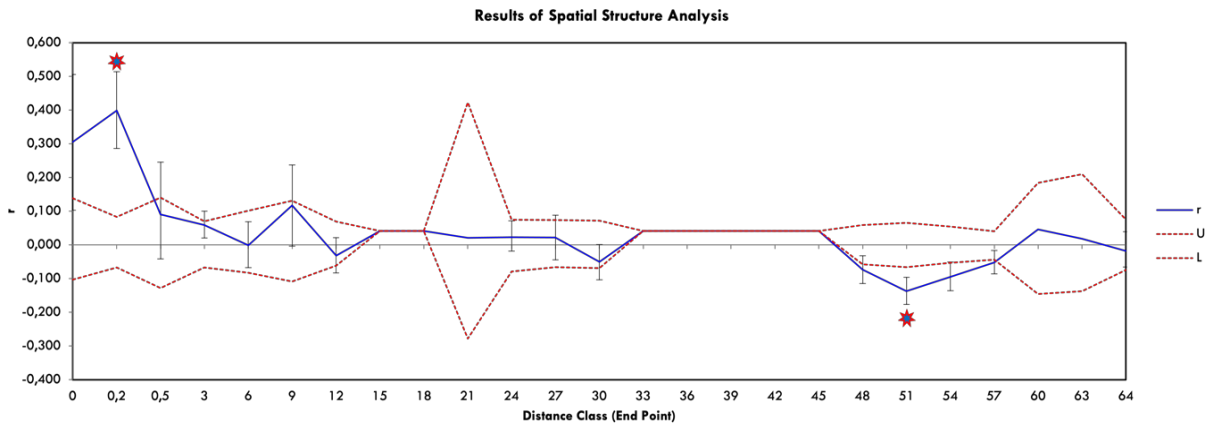
Afterwards, the test was performed for males only, where no correlograms showed significance and the gaps were different (from about 13 to 45 km) (Figure 3.25 d), e), f)) All showed an expected degree of similarity at a shorter distance, with an expected level of dissimilarity at greater distances. This indicates a more random pattern of distribution of individuals across the landscape – a predictable result for the dispersing sex.

In the case of the females (Figure 3.25 g), h), i)), only the correlogram of 5 km distance interval (Figure 3.25 i)) was considered significant (p value = 0.006) and the gaps were similar to the ones of the whole population, which is probably due to the fact that they compose more than half of the total samples. Females had a high degree of similarity at 0.2 km (r = 0.429) and some degree of dissimilarity at 30 (r = -0.085) and 55 km (r = -0.185). This is expected from individuals that tend to stay in their home group after reaching sexual maturity, although we could not confirm female-biased dispersal with our dataset.

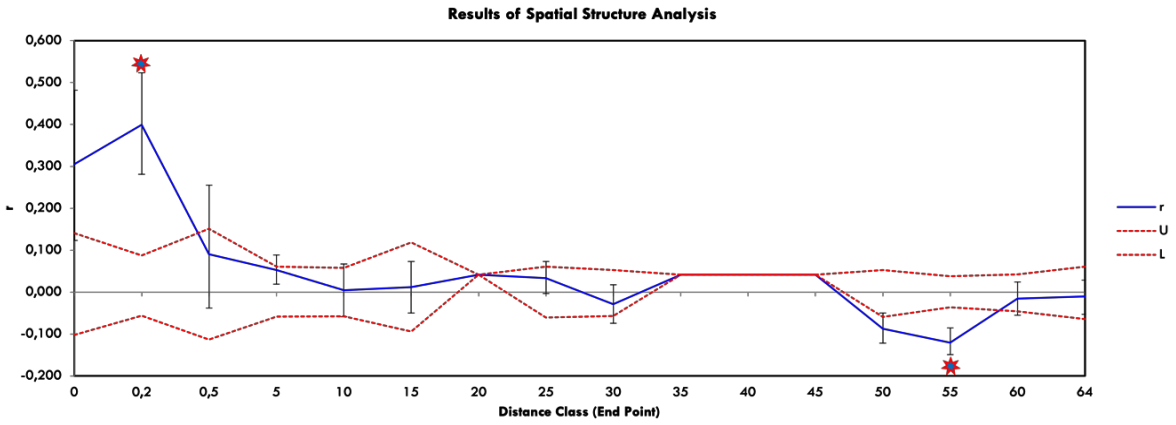
a)


Results of Spatial Structure Analysis
r
0,600
0,500
0,400
0,300
0,200
0,100
0,000
-0,100
-0,200
-0,300
-0,400
0 0,2 0,5 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61 63 64
Distance Class (End Point)
r
U
L

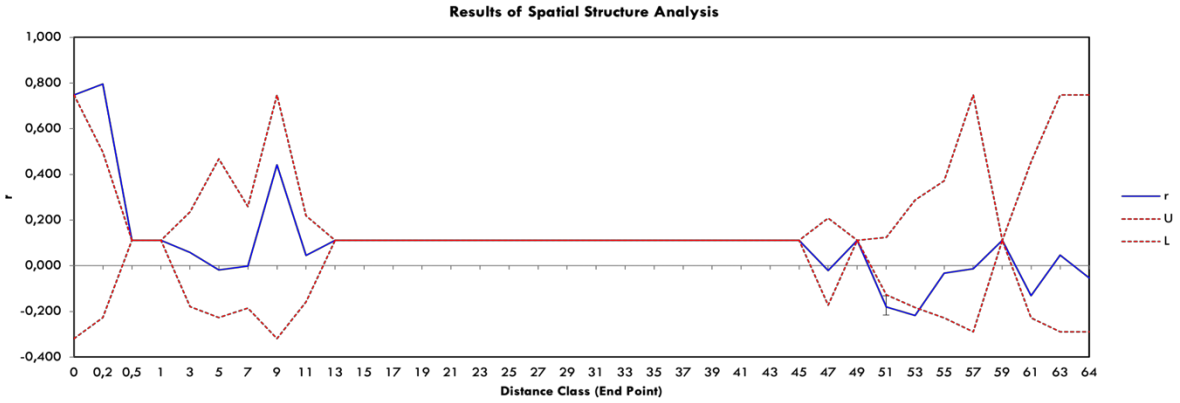
b)


Results of Spatial Structure Analysis
r
0,600
0,500
0,400
0,300
0,200
0,100
0,000
-0,100
-0,200
-0,300
-0,400
0 0,2 0,5 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60 63 64
Distance Class (End Point)
r
U
L

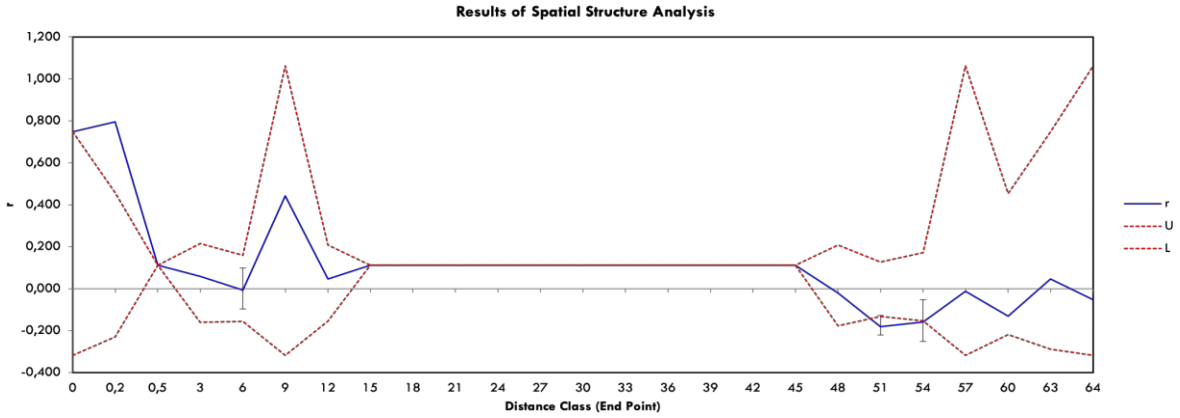
c)


Results of Spatial Structure Analysis
r
0,600
0,500
0,400
0,300
0,200
0,100
0,000
-0,100
-0,200
0 0,2 0,5 5 10 15 20 25 30 35 40 45 50 55 60 64
Distance Class (End Point)
r
U
L

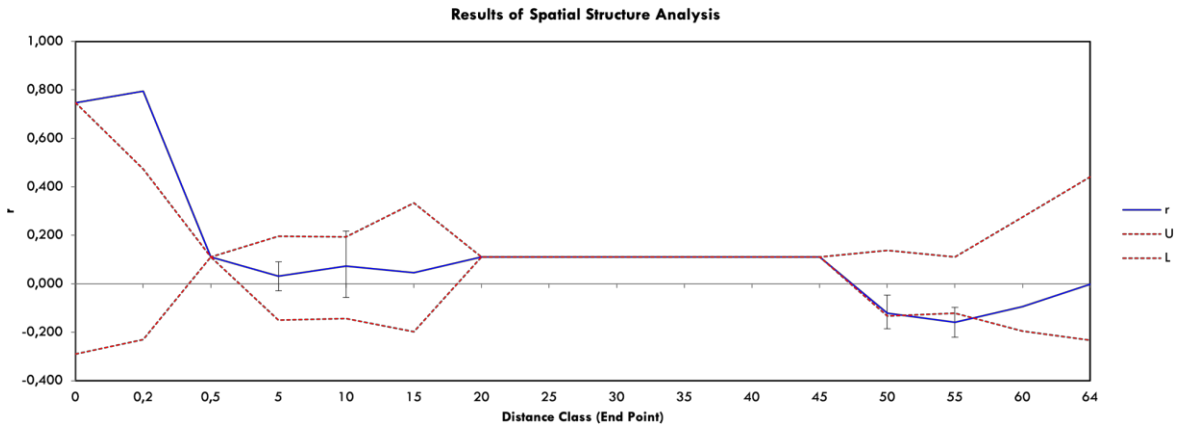
d)


Results of Spatial Structure Analysis
r
1,000
0,800
0,600
0,400
0,200
0,000
-0,200
-0,400
0 0,2 0,5 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61 63 64
Distance Class (End Point)
r
U
L

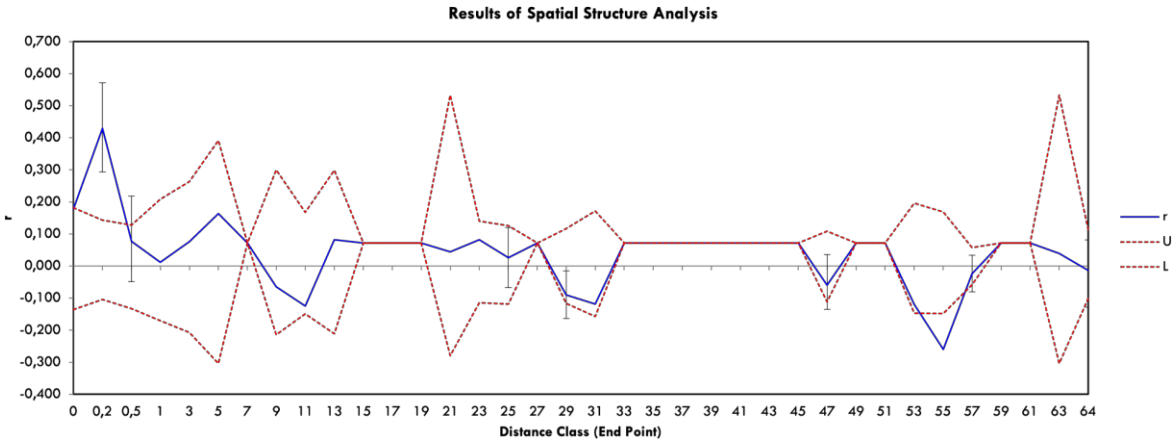
e)


Results of Spatial Structure Analysis
r
1,200
1,000
0,800
0,600
0,400
0,200
0,000
-0,200
-0,400
0 0,2 0,5 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60 63 64
Distance Class (End Point)
r
U
L

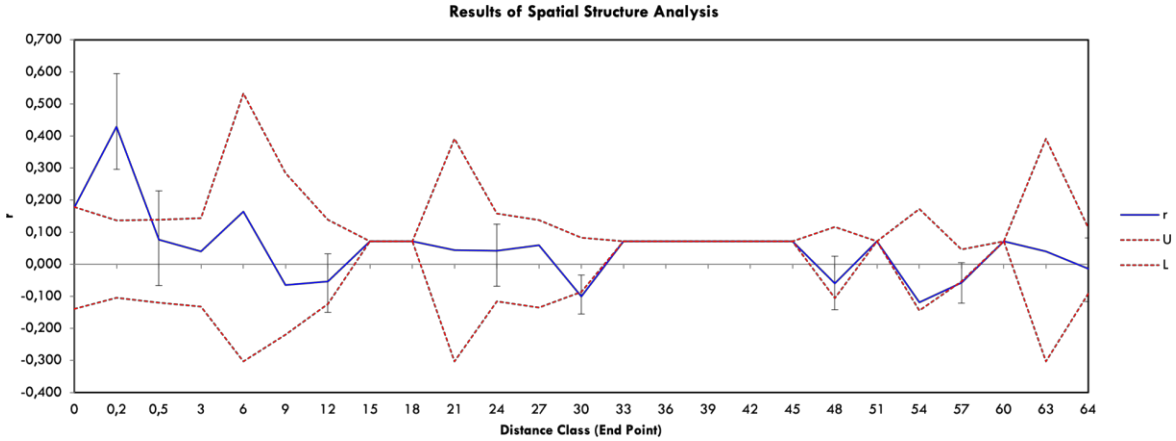
f)


Results of Spatial Structure Analysis
r
1,000
0,800
0,600
0,400
0,200
0,000
-0,200
-0,400
0 0,2 0,5 5 10 15 20 25 30 35 40 45 50 55 60 64
Distance Class (End Point)
r
U
L

g)



h)



i)

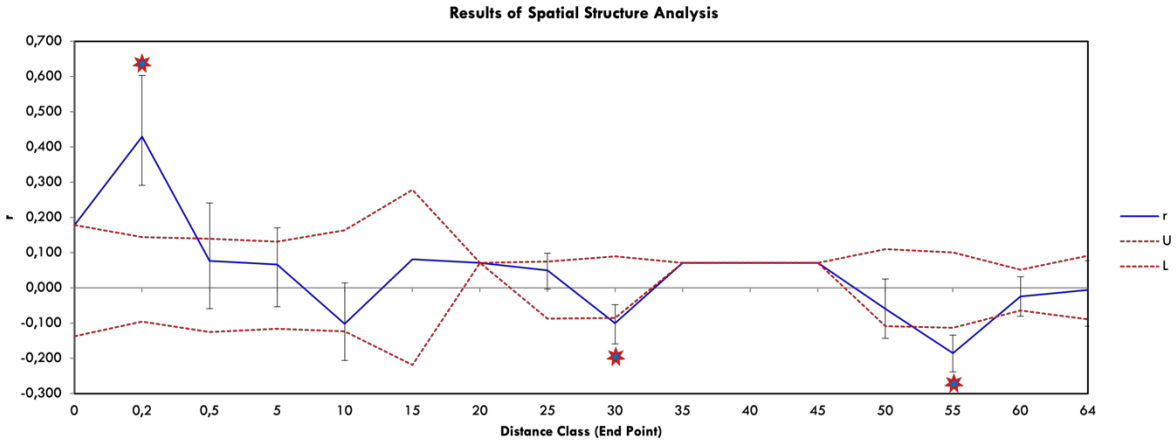


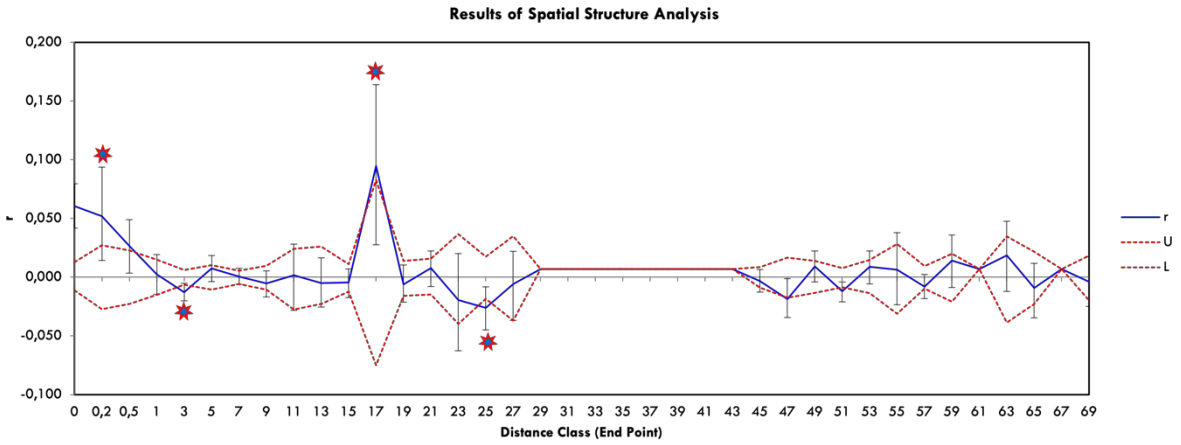
Figure 3.25 Spatial autocorrelation analysis correlograms for the BWC (N=25) population. The y-axis represents the autocorrelation coefficient (r) between genetic and geographic distances, represented by the blue line. The x-axis represents the geographic distance at the distance classes (km, end point). U and L are upper and lower limits of the 95% confidence points under the null hypothesis of random distribution of genotypes across the landscape. Error bars represent 95% confidence intervals around each mean autocorrelation coefficient. Significant pairwise genetic distances are outside the dashed red lines. Only the significant correlograms have an asterisk to aid in the identification of the significant deviations. Whole population: a) Correlogram with 2 km distance class; b) Correlogram with 3 km distance class; c) Correlogram with 5 km distance class. Males: d) Correlogram with 2 km distance class e) Correlogram with 3 km distance class; f) Correlogram with 5 km distance class. Females: g) Correlogram with 2 km distance class h) Correlogram with 3 km distance class; i) Correlogram with 5 km distance class. All correlograms were produced with GenAlEx v. 6.5.

As for the analyses for the RC population, the correlograms with distance intervals of 2 km (Figure 3.26 a)), 3 km (Figure 3.26 b)) and 5 km (Figure 3.26 c)) were found to be significant (p values of 0.009, 0.001 and 0.001 respectively). Individuals were significantly positively related at the distance of 0 km (r = 0.060) in all the different correlograms (2, 3 and 5 km distance intervals). At the finest scale of 2 km distance intervals, the relatedness deviated from expectations after the 0 km mark and presented as highest at 17 km (r = 0.095). At the distance of 3 and 25 km, relatedness dipped slightly than expected (r = -0.013 and -0.026 respectively). In the 3 km distance interval, some degree of dissimilarity between dyads was found again at 3 (r = -0.010) and 27 km (r = -0.022). Finally, at the 5 km distance interval, some of these slight deviations of relatedness disappeared excluding the positive relatedness at the 0 km distance and a slight negative deviation from relatedness at 5 km (r = -0.007). The gaps of the blocks appear at approximately 30 to 45 km on the different correlograms (whole population, males, and females), probably due to the separation between the Gola South and Central blocks of the park. After those gaps, no unexpected significant relatedness, positive or negative, appeared in any of the correlograms of the whole population. These results seem to suggest some complexity in the distribution of individuals between the neighboring groups starting at the 3 km distance with some slight deviations from what is expected at random, as well as a predictable high degree of relatedness between dyads within the social group.

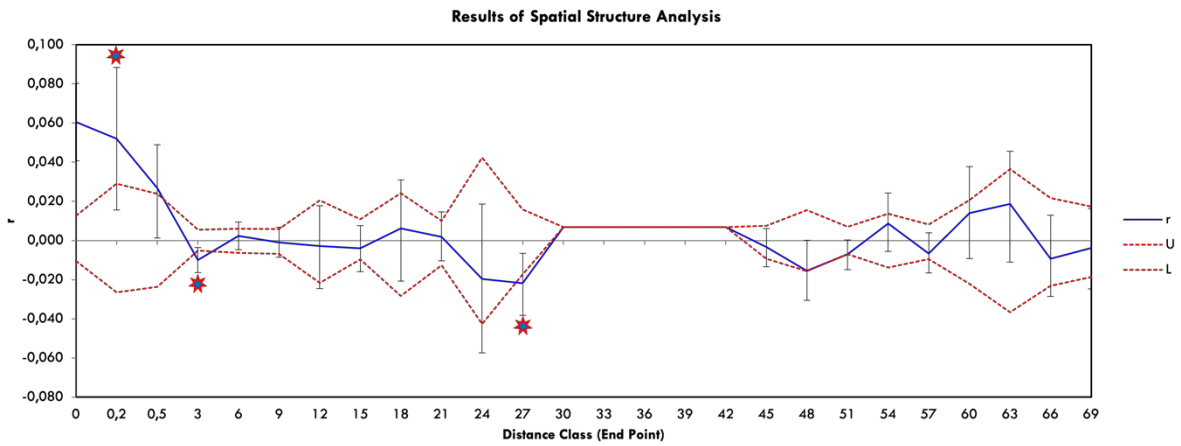
The analyses for males of RC had no significance at the 2 km distance class (Figure 3.26 d)) (p value = 0.044) but were significant at 3 km (Figure 3.26 e)) (p value = 0.009) and at 5 km (Figure 3.26 f)) (p value = 0.001) distance intervals. All the tests showed that the individuals were significantly positively related at the 0.2 km mark (r = 0.116). Much like in the whole population correlograms, the test with the 2 km distance interval showed a significative negative deviation from normal relatedness at the 3 (r = -0.033) and 25 km (r = -0.055) marks. At the 3 km distance interval, the correlogram still presented a significantly negative relatedness at the 3 km mark (r = -0.023), while the next significant negative relatedness was detected at the 27 km mark (r = -0.055). Lastly, at the 5 km distance interval correlogram the significant negative relatedness appeared again at the 5 km mark (r = -0.019), at the 25 km mark (r = -0.030) and at the 55 km mark (r = -0.036). These results seem to indicate that males from the population are more related to each other at the immediately adjacent social group level and that they are somewhat dissimilar to the males from the farthest social groups – an expected result for the more philopatric sex.

When I conducted the same test for the RC females, their correlograms also presented non-significant results for all the distance intervals tested – 2 km (Figure 3.26 g)) (p value = 0.030), 3km (Figure 3.26 h)) and 5km (Figure 3.26 i)) (p value = 0.001) and showed significant positive relatedness between dyads at 0 km (r = 0.076). Significantly non-related dyads were found at the 3 km (r = -0.015) and 57 km (r = -0.023) marks of the 2 km distance interval correlogram. At the 3 km distance interval correlogram, significantly non-related dyads were found again at the 3 km mark (r = -0.013), but the trend disappeared at the 5 km distance interval correlogram. Thus, the females may be moving to the farthest social groups and not so much to the immediately adjacent ones (3 km), being somewhat unrelated to females in the most distant regions from their home groups.

a)


Results of Spatial Structure Analysis
r
0,200
0,150
0,100
0,050
0,000
-0,050
-0,100
0 0,2 0,5 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61 63 65 67 69
Distance Class (End Point)
r
U
L

b)


Results of Spatial Structure Analysis
r
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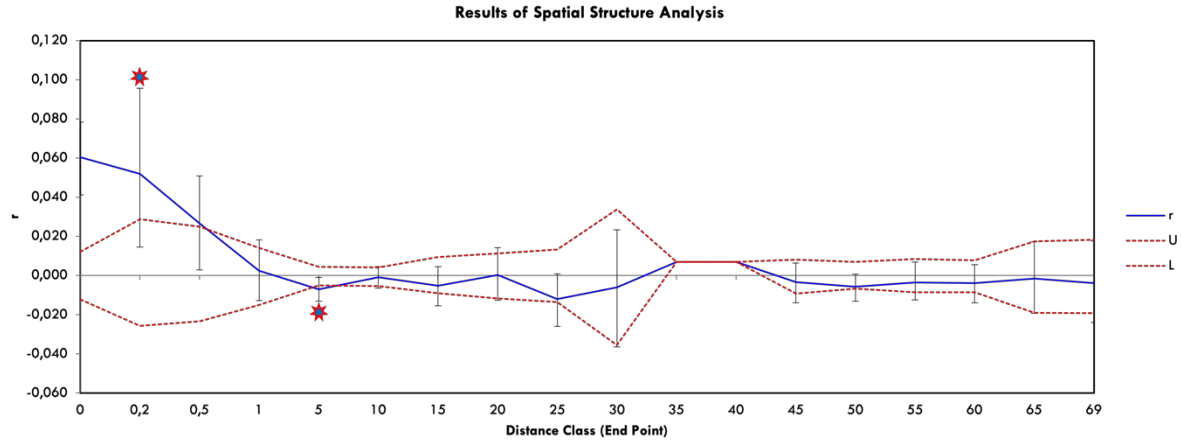
0,080
0,060
0,040
0,020
0,000
-0,020
-0,040
-0,060
-0,080
0 0,2 0,5 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60 63 66 69
Distance Class (End Point)
r
U
L

c)


Results of Spatial Structure Analysis
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0,040
0,020
0,000
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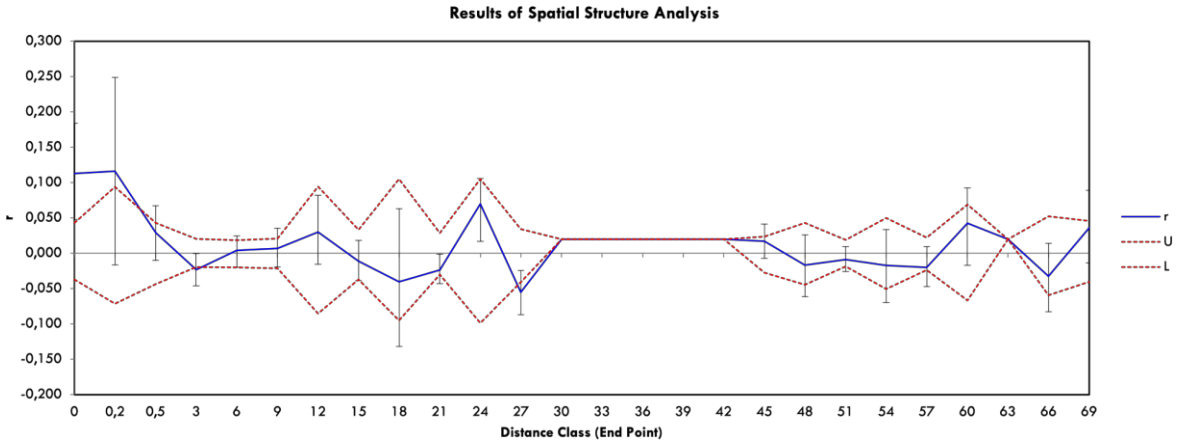
-0,040
-0,060
0 0,2 0,5 1 5 10 15 20 25 30 35 40 45 50 55 60 65 69
Distance Class (End Point)
r
U
L

d)


Results of Spatial Structure Analysis
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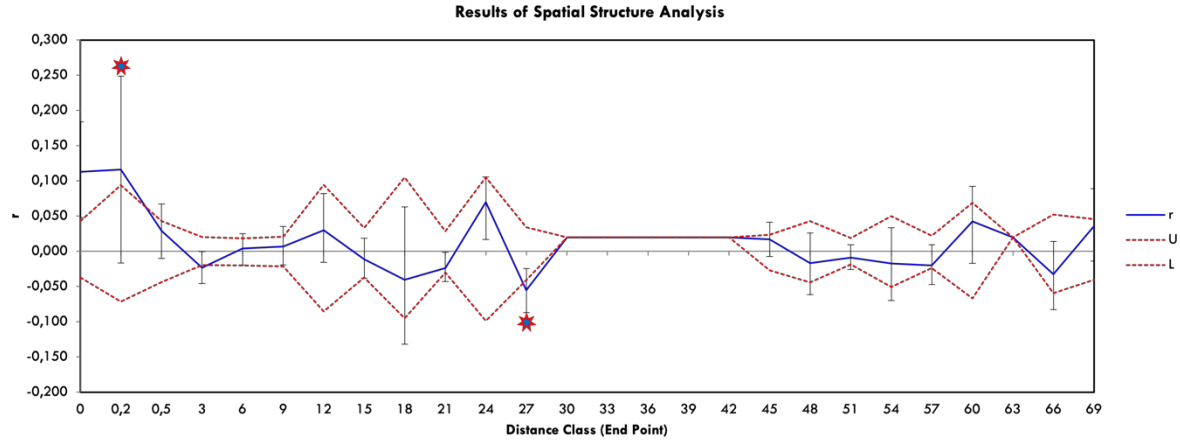
0,150
0,100
0,050
0,000
-0,050
-0,100
-0,150
-0,200
0 0,2 0,5 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60 63 66 69
Distance Class (End Point)
r
U
L

e)


Results of Spatial Structure Analysis
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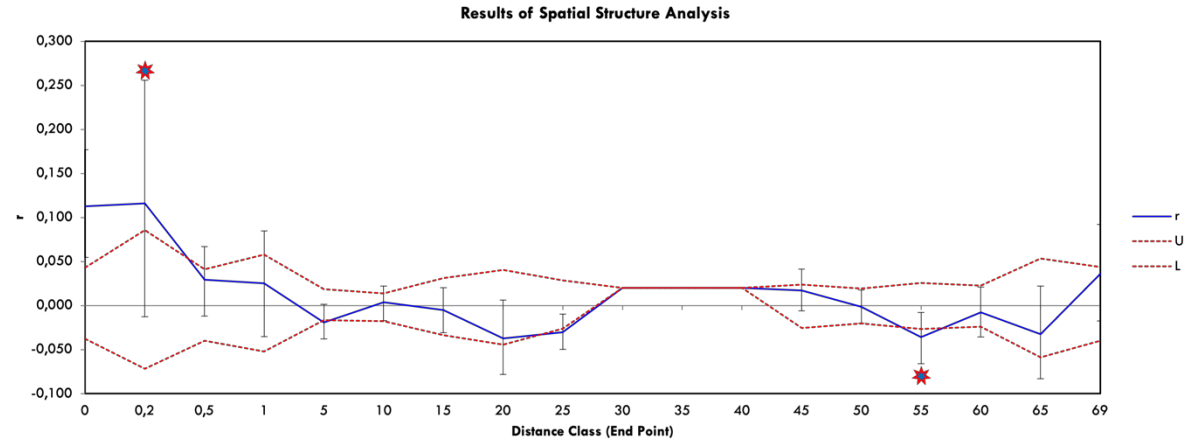
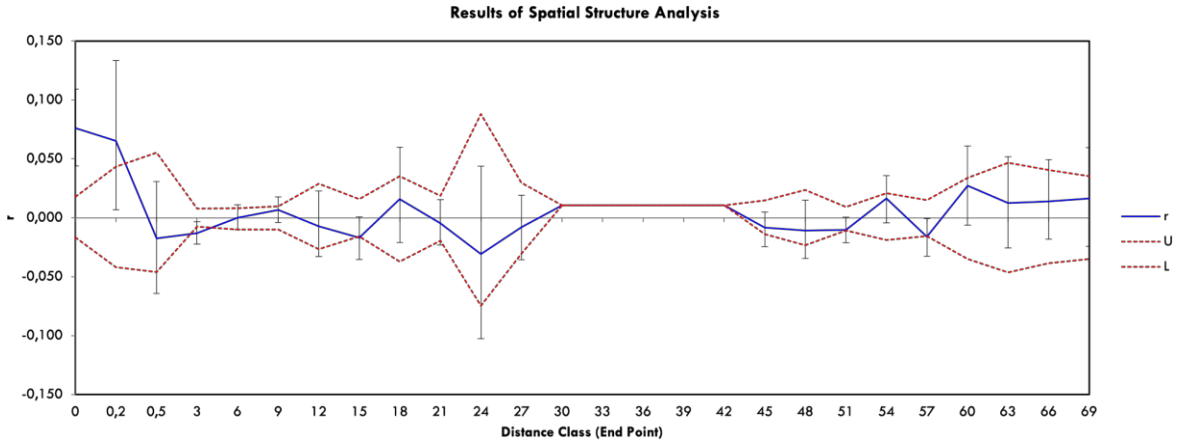
0,250
0,200
0,150
0,100
0,050
0,000
-0,050
-0,100
-0,150
-0,200
0 0,2 0,5 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60 63 66 69
Distance Class (End Point)
r
U
L

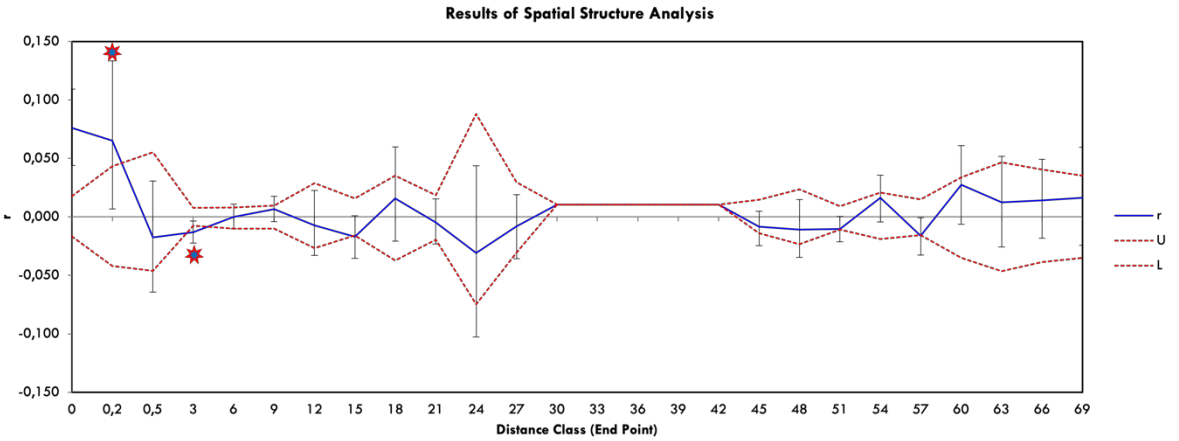
f)


Results of Spatial Structure Analysis
r
0,300
0,250
0,200
0,150
0,100
0,050
0,000
-0,050
-0,100
0 0,2 0,5 1 5 10 15 20 25 30 35 40 45 50 55 60 65 69
Distance Class (End Point)
r
U
L

g)



h)



i)

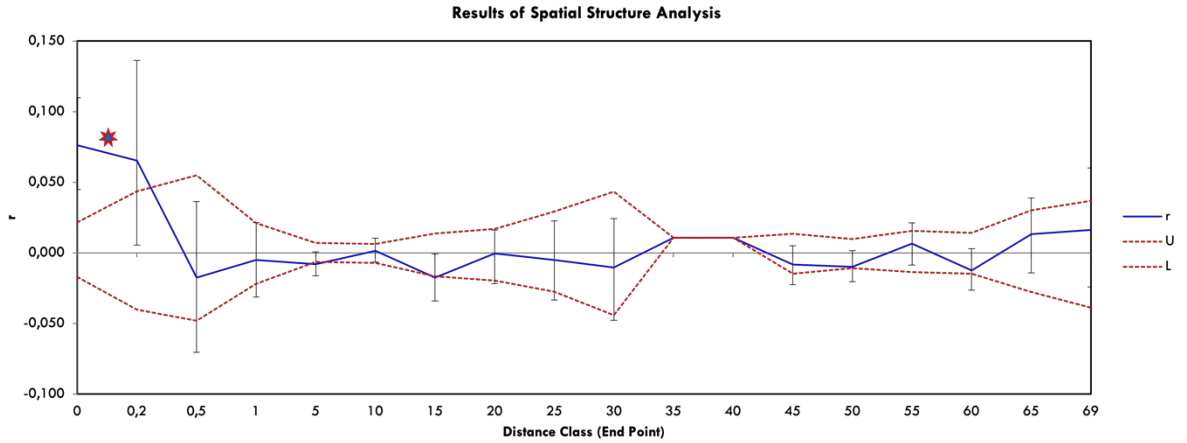


Figure 3.26 Spatial autocorrelation analysis correlograms for the RC (N=146) population. The y-axis represents the autocorrelation coefficient (*r*) between genetic and geographic distances, represented by the blue line. The x-axis represents the geographic distance at the distance classes (km, end point). U and L are upper and lower limits of the 95% confidence points under the null hypothesis of random distribution of genotypes across the landscape. Error bars represent 95% confidence intervals around each mean autocorrelation coefficient. Significant pairwise genetic distances are outside the dashed red lines. Only the significant correlograms have an asterisk to aid in the identification of the significant deviations. Whole population: a) Correlogram with 2 km distance class; b) Correlogram with 3 km distance class; c) Correlogram with 5 km distance class. Males: d) Correlogram with 2 km distance class e) Correlogram with 3 km distance class; f) Correlogram with 5 km distance class. Females: g) Correlogram with 2 km distance class h) Correlogram with 3 km distance class; i) Correlogram with 5 km distance class. Produced with GenAlEx v. 6.5.

3.5.2 Structure Tessellation

Firstly, Kmax – the maximum number of clusters that the population can have – is to be selected by observing of the stabilization of the number of K on the DIC values. This plot has the DIC of every number of clusters (K) from the model without admixture, and once the curve stabilizes on one number, then we'll know the Kmax. For BWC, this happened at K=7 for the 25 individuals' dataset (Figure 3.27), at K=6 for the 18 individuals' dataset (Figure 3.28) and for RC it happened at K=9 (Figure 3.29), thus selecting the maximum number of clusters for each population.

Since Kmax is usually not representative of the true number of clusters in a population, it was important to look at the admixture proportions for every number of K. For the 25 individual's database of BWC, there seemed to be two main clusters present (first mostly blue, second mostly yellow) and a third with only two individuals (mostly green, with a bit of yellow and blue) (Figure 3.30 a) and b) respectively). In the 18 individual's dataset, the individuals seem to belong to one cluster of mostly green and another one having elements of the three clusters in each individual (green, yellow and blue) (Figure 3.31 a) and b) respectively). In the case of RC, all the admixture plots showed a very homogenous population (Figure 3.32), with two main outlying individuals: BW050901 and RC051918. The examination of the former presented four rare alleles, two private alleles and one rare genotype. The latter sample contained five rare alleles, one private allele and an infrequent combination. These can be sufficient causes for their exceptional position in the admixed population. We can therefore conclude that, according to the admixture proportions of the individuals, the BWC with 25 samples dataset is best explained by K=3, the BWC with 18 samples dataset is best explained by K=2 and the RC dataset is also best explained by K=2.

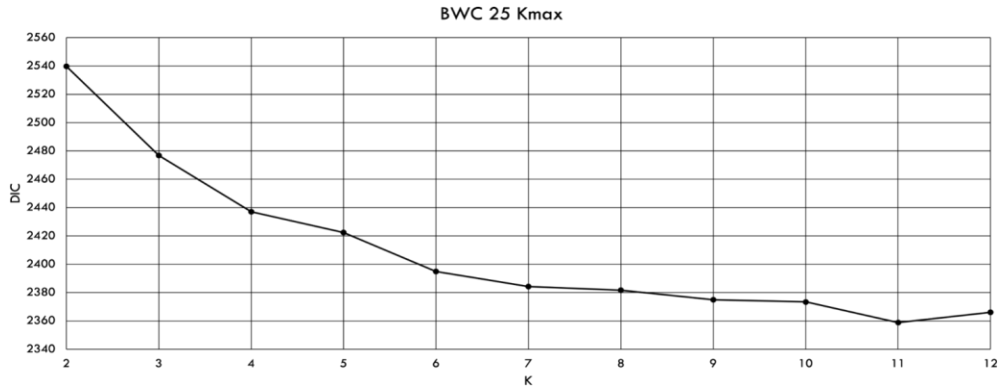


Figure 3.27 Plot of DIC (y-axis) against K (x-axis). The stabilization of the line on one value of K indicates the best fitting number of Kmax, or the maximum number of genetic clusters the population (BWC, N=25) can have. Produced with TESS v. 2.3.1., CLUMPP v.1.1.2. and RStudio v. 1.4.1106.

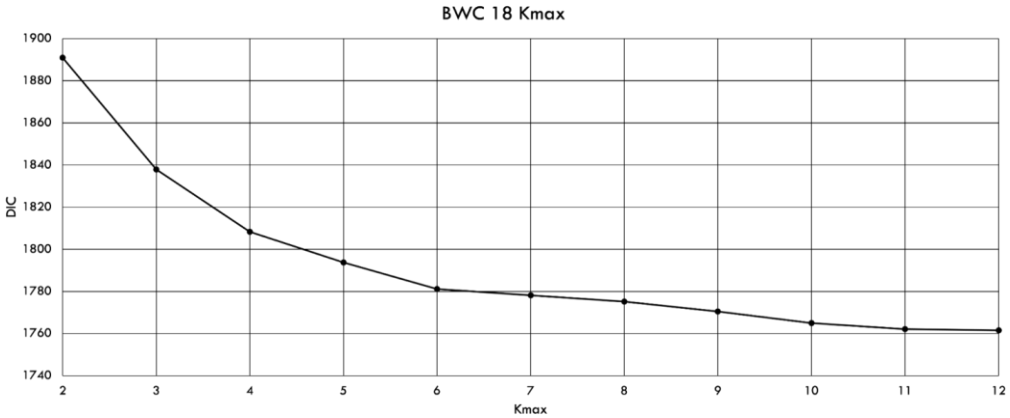


Figure 3.28 Plot of DIC (y-axis) against K (x-axis). The stabilization of the line on one value of K indicates the best fitting number of Kmax, or the maximum number of genetic clusters this population (BWC, N=18) can have. Produced with TESS v. 2.3.1, CLUMPP v.1.1.2. and RStudio v. 1.4.1106.

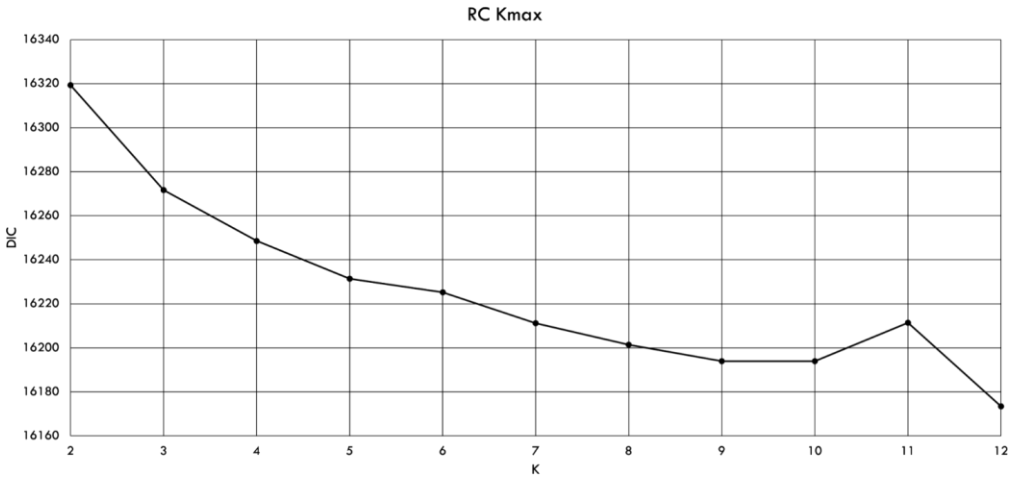


Figure 3.29 Plot of DIC (y-axis) against K (x-axis). The stabilization of the line on one value of K indicates the best fitting number of Kmax, or the maximum number of genetic clusters this population (RC, N=146 individuals) can have. Produced with TESS v. 2.3.1, CLUMPP v.1.1.2. and RStudio v. 1.4.1106.

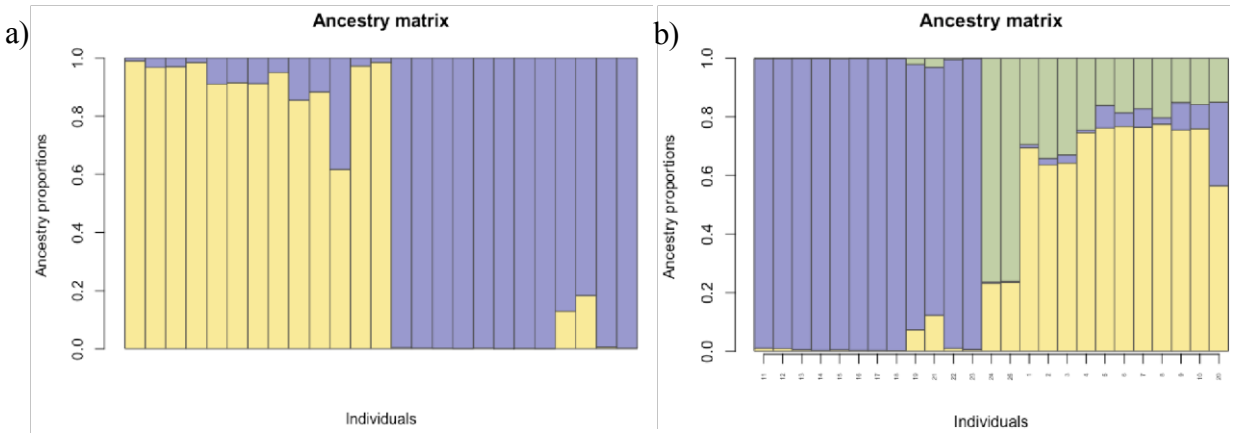


Figure 3.30 Admixture proportions of individuals (vertical lines) in the BWC population with 25 individuals representing K=2 and K=3 – a) and b) respectively. Produced with TESS v. 2.3.1, CLUMPP v.1.1.2. and RStudio v. 1.4.1106.

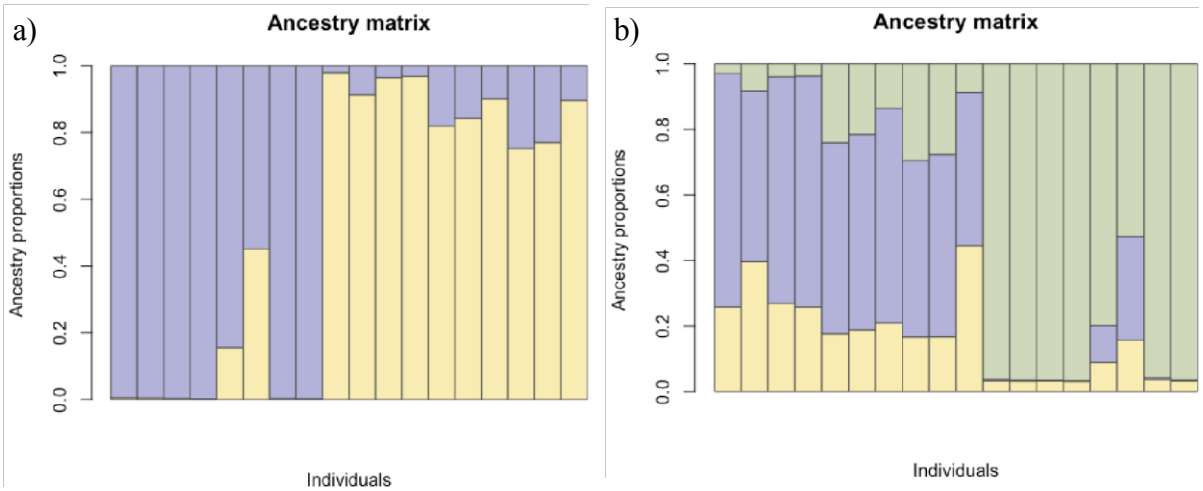


Figure 3.31 Admixture proportions of individuals (vertical lines) in the BWC population with 18 individuals representing K=2 and K=3 – a) and b) respectively. Produced with TESS v. 2.3.1, CLUMPP v.1.1.2. and RStudio v. 1.4.1106.

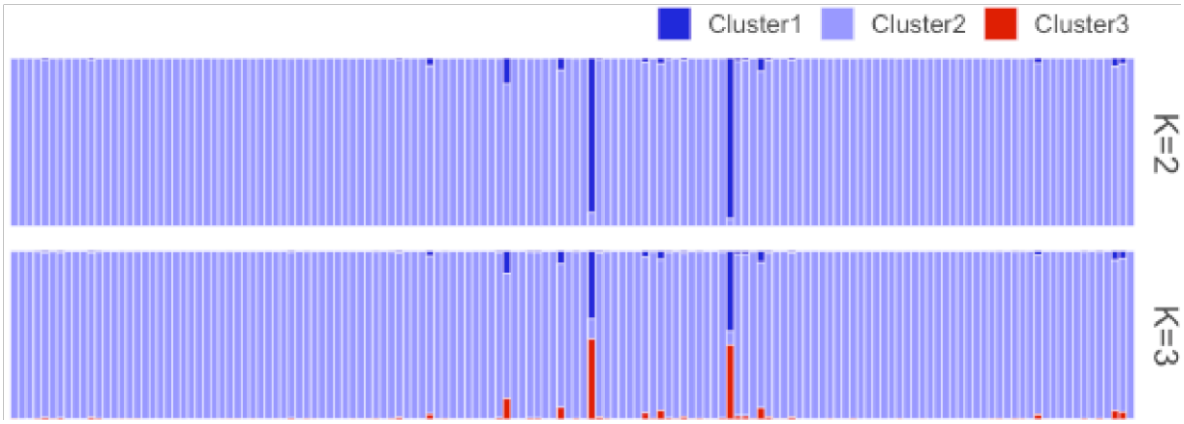


Figure 3.32 Admixture proportions of individuals (vertical lines) in the RC population representing K=2 and K=3. Produced with TESS v. 2.3.1, CLUMPP v.1.1.2. and RStudio v. 1.4.1106.

To obtain a clearer picture of the admixture of the populations, the clusters were superimposed on the map of the study area. In the BWC population with 25 individuals, a division between clusters appeared in the middle of Gola South at the K=2 map (Figure 3.33). At the K=3 map (Figure 3.34), another small cluster started to appear between them, insisting on a genetic division in that location of the park (Annex 7.5, Figure 7.7 c)). All the other maps with the different numbers of K did not vary apart from this trend of one cluster at Gola South connecting to Tiwai, a small one in the middle of Gola South and another one starting to the right of this block and continuing to the Center-North block. The only exceptions appeared on the K=5 map (Annex 7.5, Figure 7.7 b)) that for the first time included Tiwai in this latter cluster and at the K=6 map (Annex 7.5, Figure 7.7 c)), where another minuscule cluster appeared next to the one that created a division between the two main clusters, but it disappeared with the increase in the number of K (Annex 7.5, Figure 7.7 d)). In the BWC population with 18 individuals (without the highly related ones), the same division happened between the two main clusters (Figure 3.35), but no more clustering was found from K=2 to K=10 (Annex 7.5, Figure 7.9). From this, it is possible to conclude that this population is best explained by K=2 with one of the clusters existing due to a set of highly related individuals. Finally, in the RC population, there appeared to be only one cluster (Figure 3.36), showing a cohesive and panmictic population from K=2 to K=10 (Annex 7.5, Figure 7.9) – suggesting an absence of substructuring.

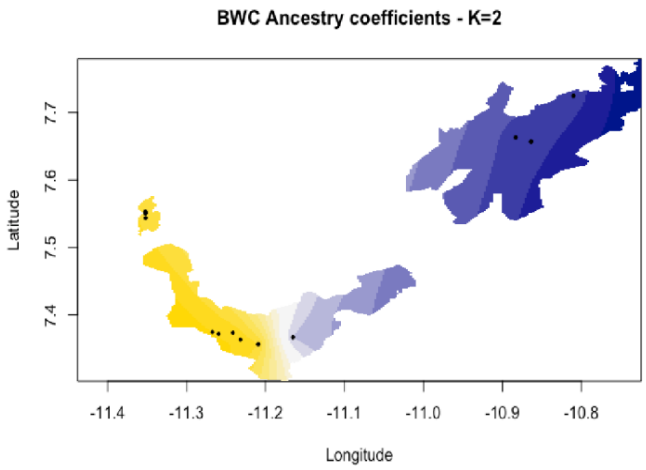


Figure 3.33 Structure tessellation superimposed on study area considering K=2 for the BWC 25 individuals' population. Produced with TESS v. 2.3.1, CLUMPP v.1.1.2. and RStudio v. 1.4.1106.

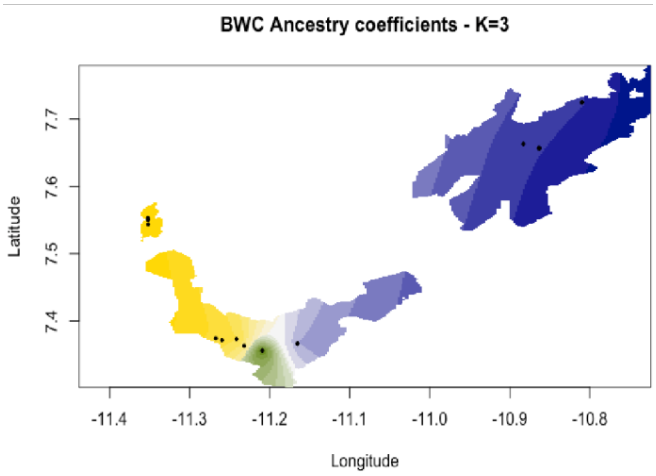


Figure 3.34 Structure tessellation superimposed on study area considering K=3 for the BWC 25 individuals' population. Produced with TESS v. 2.3.1, CLUMPP v.1.1.2. and RStudio v. 1.4.1106.

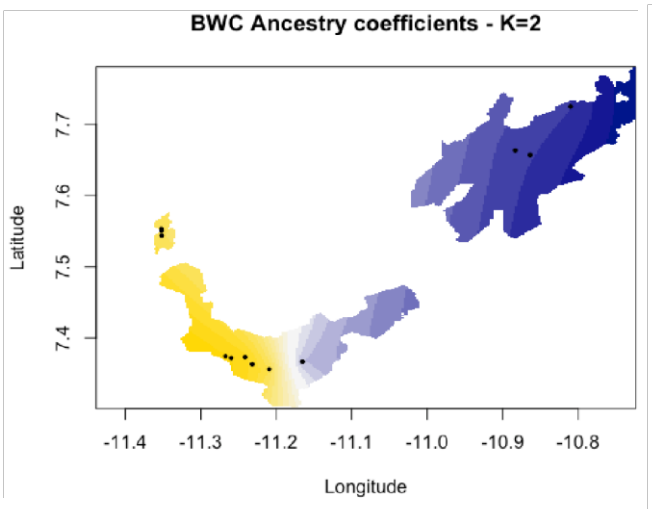


Figure 3.35 Structure tessellation superimposed on study area considering K=2 for the BWC 18 individuals' population.Produced with TESS v. 2.3.1, CLUMPP v.1.1.2. and RStudio v. 1.4.1106.

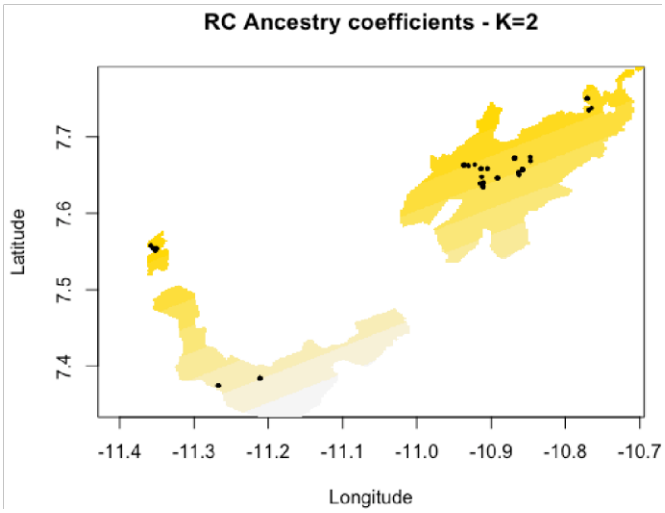


Figure 3.36 Structure tessellation superimposed on study area considering K=2 for the RC population. Produced with TESS v. 2.3.1, CLUMPP v.1.1.2. and RStudio v. 1.4.1106.

3.6 Sex-biased Dispersal

In order to identify the main dispersing sex in the population, I proceeded to the calculation of mean corrected assignment indices (mAI_C) for males and females. The dispersing sex should present a negative mAI_C, while the more philopatric sex shall present a positive mAI_C. The corrected Assignment Index (mAI_C) was calculated for 15 females (1.043) and 10 males (-1.565) of the whole BWC population (Figure 3.37), revealing non-significant sex-biased dispersal patterns (p value = 0.181). However, since most of the dispersal events occur between social groups within a population, I investigated the dispersal patterns within forest blocks and transects, whenever the sample size allowed. The test was then performed for 5 females (-0.350) and 5 males (0.350) from Gola Central (Figure 3.38), 4 females (0.800) and 5 males (-0.640) from Gola South (Figure 3.39) and 4 females (-0.714) and 2 males (1.427) from the Transect 44 (situated in Gola Central) (Figure 3.40). I found no significant difference between the sexes for any of the tests conducted, suggesting an absence of sex-biased dispersal for this population.

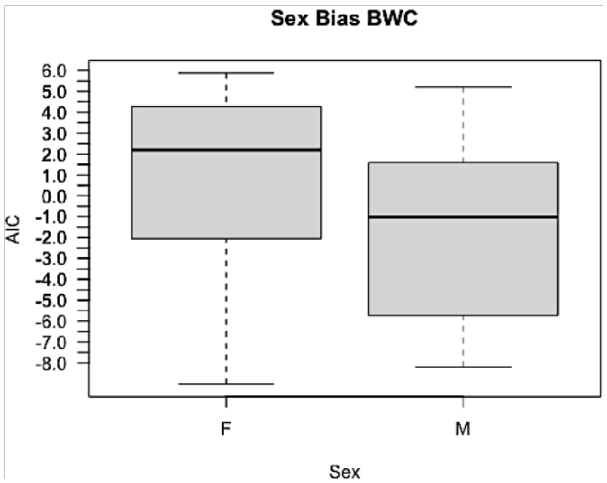


Figure 3.37 Boxplot of corrected Assignment Indexes and respective error bars, for males and females of the BWC population with 25 individuals. Produced in RStudio v. 1.4.1106.

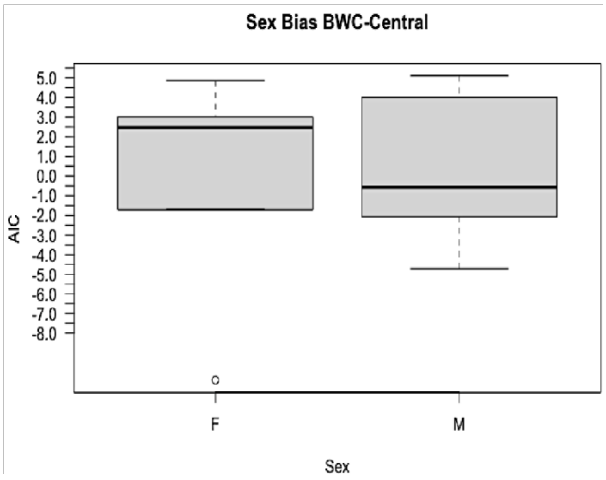


Figure 3.38 Boxplot of corrected Assignment Indexes and respective error bars, for males and females of the BWC individuals' samples located in Gola Central. Produced in RStudio v. 1.4.1106.

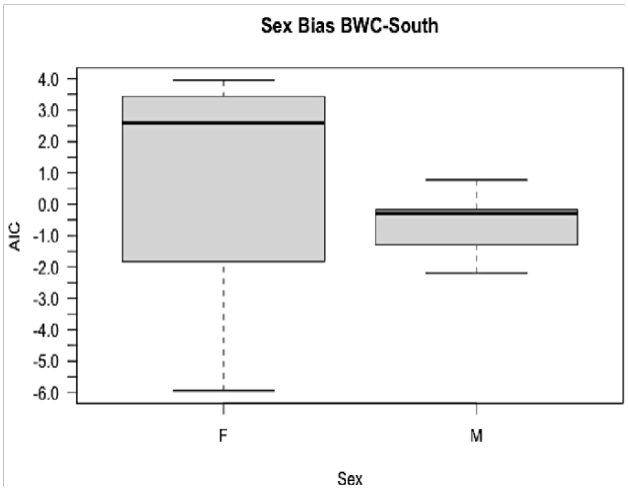


Figure 3.39 Boxplot of corrected Assignment Indexes and respective error bars, for males and females of the BWC individuals' samples located in Gola South. Produced in RStudio v. 1.4.1106.

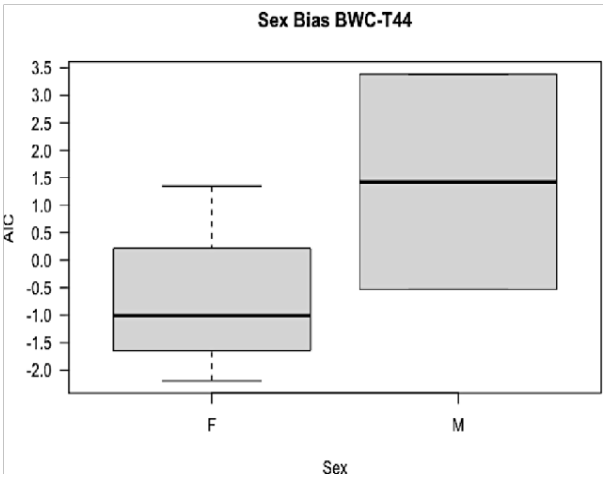


Figure 3.40 Boxplot of corrected Assignment Indexes and respective error bars, for males and females of the BWC individuals' samples in Transect 44. Produced in RStudio v. 1.4.1106.

As for RC, the mAI_c was calculated for 96 females (-0.189) and 52 males (0.353) of the whole population, also revealing an absence of sex-biased dispersal for this population (p value of 0.614, Figure 3.41). On the Tiwai island, there were 6 females and 8 males, with mAI_c of -2.248 and 1.686 respectively (Figure 3.42).

Next, the population of the GRNP was separated into blocks for the same analysis. In the South block, the 14 females had an mAI_c of 1.000 and the 7 males had a mAI_c of -1.999 (Figure 3.43 a)). The test was also performed on 68 females (-0.236) and 32 males (0.502) from Gola Central (Figure 3.43 b)), then on 7 females (0.017) and 4 males (-0.030) from Gola Central-North (Figure 3.43 c)) and finally, on these two blocks together (Gola Central and Central-North, Figure 3.43 d)), with 75 females (-0.341) and 36 males (0.709).

For the transects scale, the RT44 – located in the Central North block – also had enough individuals for the test, with 8 females (-0.533) and 4 males (1.066) (Figure 3.43 e)). In the Central block, the T37 (Figure 3.43 f)) has 17 females and 7 males with mAI_c of 0.419 and -1.0171 respectively. Finally, the RT10 of the Gola South block had 12 females with an mAI_c of 1.114 and 6 males with mAI_c of -2.22859 (Figure 3.43 g)). The p values of all tests conducted for this species yielded non-significant results (the lowest p value being 0.122 for the Tiwai island samples), thus suggesting an absence of sex-biased dispersal in this population.

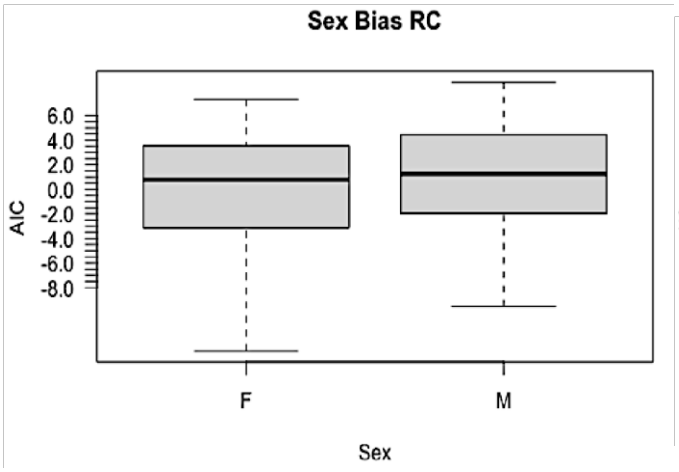


Figure 3.41 Boxplot of corrected Assignment Indexes and respective error bars, for males and females of the whole RC population. Produced in RStudio v. 1.4.1106.

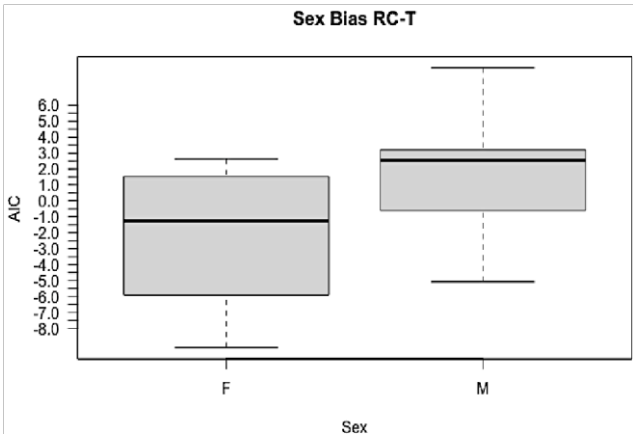
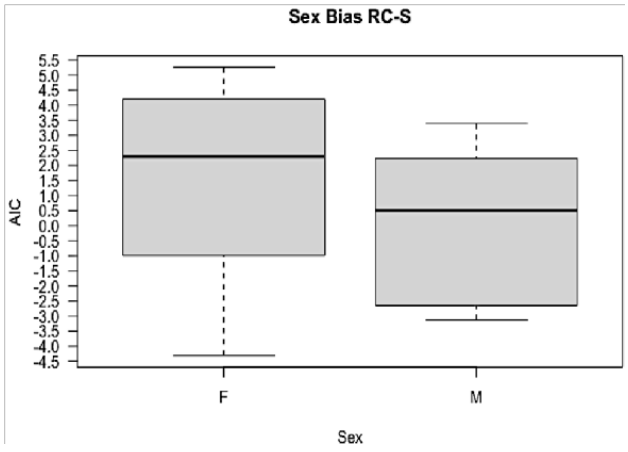
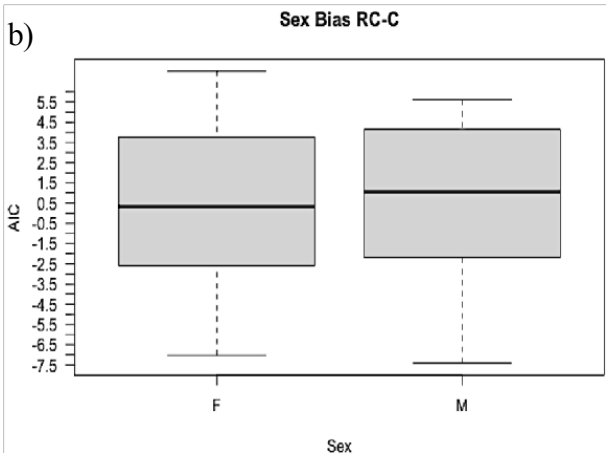


Figure 3.42 Boxplot of corrected Assignment Indexes and respective error bars, for males and females of samples encountered on the Tiwai Island. Produced in RStudio v. 1.4.1106.

a)



b)



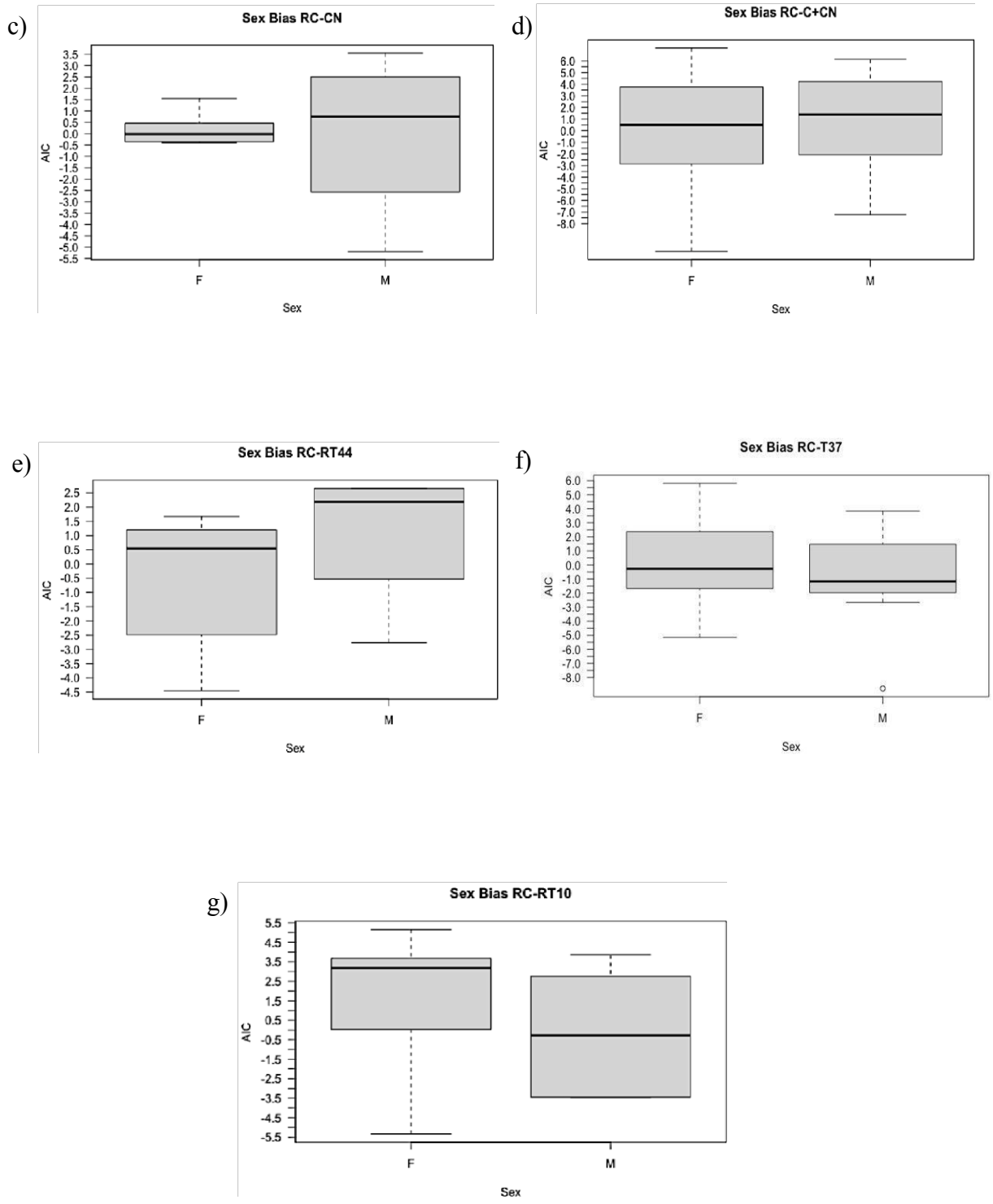


Figure 3.43 Boxplots of corrected Assignment Indexes and respective error bars, for males and females sampled in: a) Gola South block; b) Gola Central block; c) Gola Central block, northern section; d) Gola Central block with the Central-North section; e) Road to Transect 44; f) Transect 37; g) Road to Transect 10. Produced in RStudio v. 1.4.1106.

3.7 Demographic History

The results from the MSVAR 1.3. program suggest the presence of a bottleneck for both species at different times. A strong bottleneck signal was found in the case of BWC (Figure 3.44), with minimal differences between the complete and "unrelated" (Annex 7.6, Figure 7.10) datasets. This indicates that kin-related structure in the BWC population probably did not induce a false demographic bottleneck signal, making the discussion of the results from the dataset with unrelated individuals irrelevant. A limited degree of overlap between the posterior distributions of N_0 and N_1 can be observed, which is confirmed by the 90% highest posterior density intervals (HPD = 90%; N_0 = 1 - 6,438 individuals, N_1 = 2,096 - 31,186 individuals, Table 3.9.). The difference between the median of the current (N_0) and of the ancestral (N_1) population size is considerable: the former ranged from 286 to 592, while the latter was one order of magnitude larger, ranging from 7,787 to 8,397. This result was further supported by the N_0/ N_1 ratio, which presented median values ranging between 0.02 and 0.06 (HPD 90 % ranging from - 3,860 to -0,236, Table 3.11) thus supporting a demographic bottleneck. As for the posterior distributions of time (T) at which the demographic collapse occurred, the median values of the four runs oscillated between 1,741 and 3,437 years ago. Just like in the graphic representation of the mean values, the 90% HPD limits varied greatly, ranging from 6 to 73,713 years ago. Acceptable convergence across the four independent runs for this database was indicated by the multivariate potential scale reduction factor being close to 1 (1.035) and by the estimates of the corrected scale reduction factors, along with their 97.5% quantiles for each parameter being <1.20. Additionally, a clear pattern of convergence of the four runs into narrow peaks illustrates this convergence. In comparison, a weaker bottleneck signal was detected in RC (Figure 3.45). A great degree of overlap between the posterior distributions for N_0 and N_1 was detected for the RC population, as evidenced by the 90 % HPD intervals (N_0 = 66 - 46,127 individuals, N_1 = 4,090 – 52,579, Table 3.12). The difference between the N_0 and N_1 medians indicated a decrease in population of one order of magnitude: the median of the current (N_0) population size ranged from 2,794 to 6,151 individuals, while median of the ancestral (N_1) population size ranged from 14,131 to 15,551 individuals. The N_0/ N_1 ratio presented median values ranging between 0.18 and 0.41, with HPD 90% values ranging from -2,415 to 0,380, also suggesting the existence of a demographic bottleneck. A wide posterior distribution for the time (T) since the demographic change occurred was observed, with medians varying from 3,663 to 8,772 years ago. The HPD 90% limits also oscillated greatly, ranging from 54 to 78,739 years ago (Table 3.12). In the case of RC, the graphical convergence of the four runs was not very defined, but still clear enough to observe the different runs converging at about the same limits (Figure 3.45). The deviation from convergence is further confirmed by the corrected scale reduction factors being higher than 1.20 for some parameters (Mean N_0 and respective 97.5% quantile and Mean T's 97.5 % quantile). Concomitantly, the Multivariate Potential Scale Reduction Factor (MPSRF) obtained through the Brooks, Gelman and Rubin Convergence diagnostic test was not as close to 1 as would be ideal (<1.20). The total number of MPSRF for the runs in this species was 1.22, while the 97.5 % quantile of Mean T and the Mean N_0 Estimate and respective 97.5 % quantile also did not reach convergence. Therefore, I can conclude that the runs for the RC did not reach convergence clearly and estimates for the time and current population size especially, are uncertain. Probably, longer runs would have to be conducted in order to reach convergence among their posterior probabilities.

Table 3.11 Posterior distributions of the current population size (N_0), ancestral population size (N_1) and the time since the demographic change occurred (T, in years), per run/scenario, of the *C. polykomos* (BWC, N=25) population. From MSVAR 1.3.

	C. polykomos	Run 1	Run 2	Run 3	Run 4
N_0	Mean	505	438	262	175
	Median	592	519	358	286
	HPD (90%)	18 – 6,438	24 – 4,865	6 – 5,184	1 – 5,254
N_1	Mean	8,624	7,999	8,068	7,946
	Median	8,397	7,991	7,970	7,787
	HPD (90%)	2,213 – 31,186	2,284 – 28,482	2,236 – 28,535	2,096 – 28,095
N_0/ N_1	Mean	0.06	0.05	0.03	0.02
	Median	0.07	0.07	0.05	0.04
	HPD (90%)	-1,222 /-1,222	-2,545 /-0,259	-3,198 /-0,294	-3,860 /-0,236
T	Mean	3,284	2,996	1,890	1,359
	Median	3,437	3,099	2,207	1,741
	HPD (90%)	74 – 73,713	115 – 53,578	32 – 54,195	6 – 51,631

Table 3.12 Posterior distributions of the current population size (N_0), ancestral population size (N_1) and the time since the demographic change occurred (T, in years), per run/scenario, of the *P. b. badius* (RC, N=146) population. From MSVAR 1.3.

	P. b. badius	Run 1	Run 2	Run 3	Run 4
N_0	Mean	5,648	2,444	2,098	4,997
	Median	6,151	2,898	2,794	5,056
	HPD (90%)	647 – 46,127	159 – 24,648	66 – 31,041	779 – 34,491
N_1	Mean	15,246	15,273	15,587	14,122
	Median	15,260	15,295	15,551	14,131
	HPD (90%)	4,410 – 50,676	4,530 – 50,425	4,471 – 52,579	4,090 – 47,885
N_0/ N_1	Mean	0.37	0.16	0.13	0.35
	Median	0.41	0.20	0.18	0.35
	HPD (90%)	-1,320 /0,380	-1,990 /0,131	-2,415 /0,204	-1,190 /0,290
T	Mean	6,484	3,709	2,894	7,973
	Median	7,283	4,326	3,663	8,772
	HPD (90%)	453 – 72,190	171 – 56,213	54 – 78,739	938 – 71,510

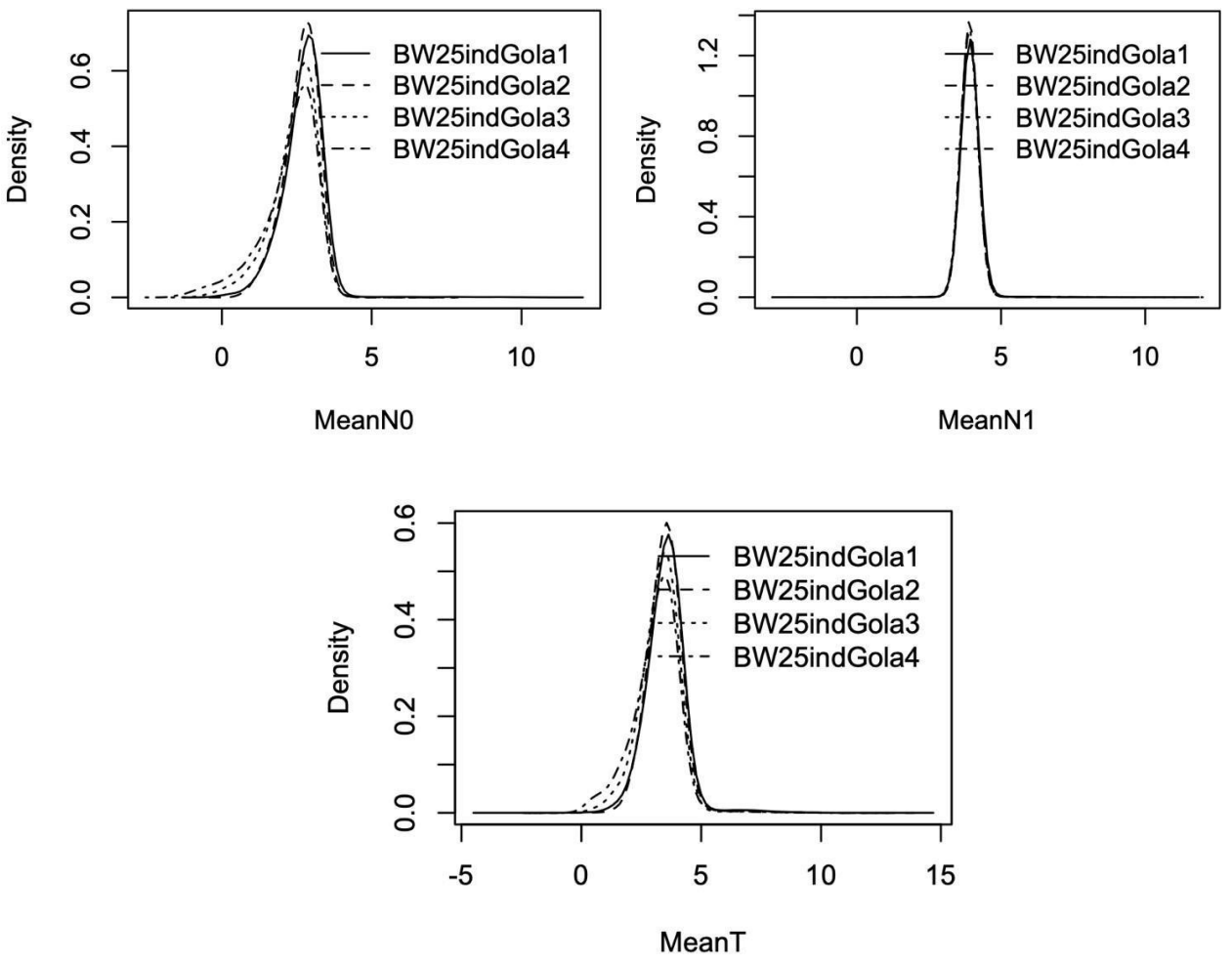


Figure 3.44 Posterior distributions of MSVAR 1.3 parameters' means in a logarithmic scale for the BWC (N=25) population: current effective population size (N_0; top left), ancestral population size (N_1; top right) and the time (T) since the occurrence of the demographic change (below). The four runs are presented and differentiated by the type of line, as reported by the inherent subtitle. Produced in RStudio v. 1.4.1106.

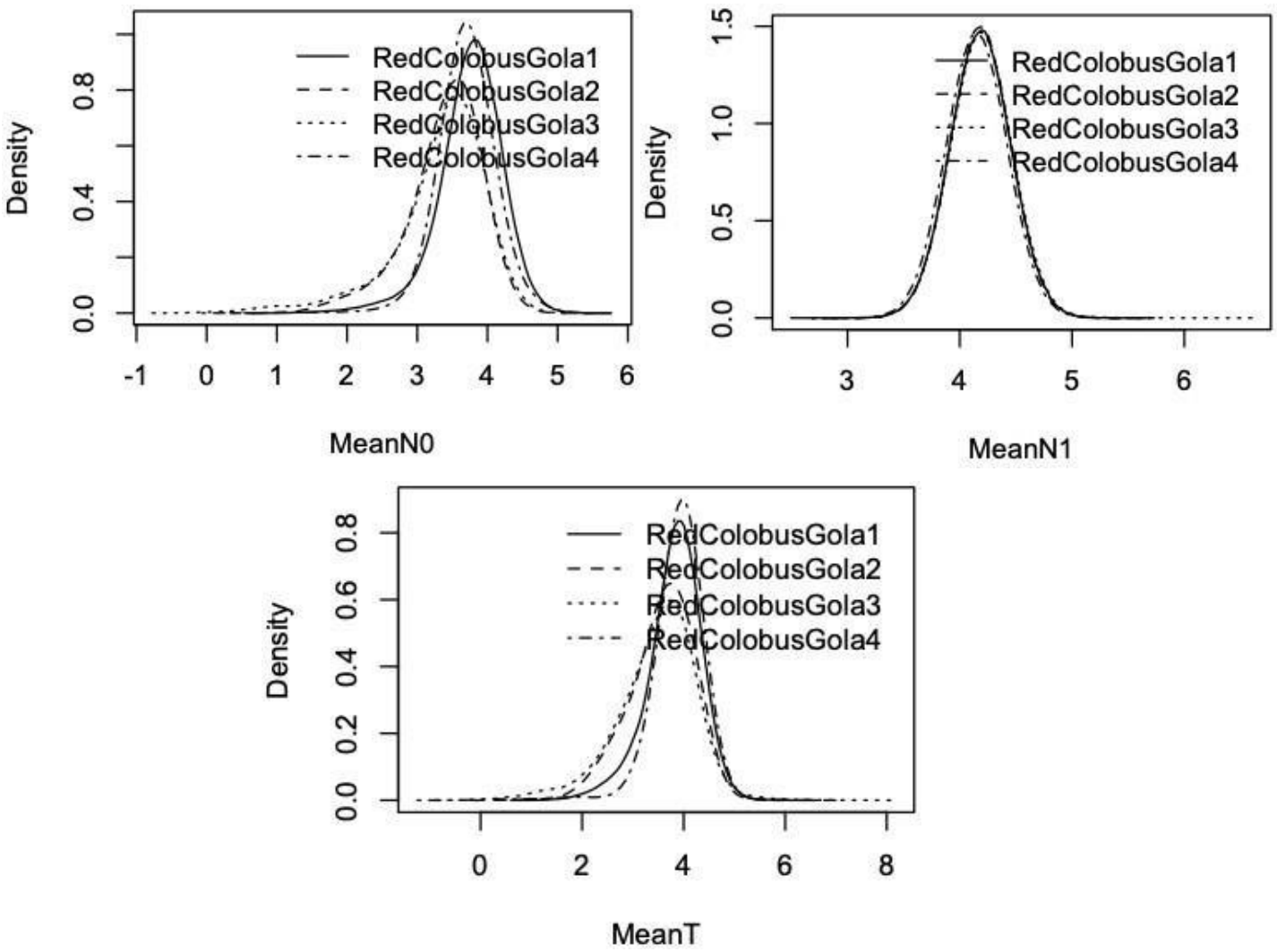


Figure 3.45 Posterior distributions of MSVAR 1.3 parameters' means in a logarithmic scale for the RC (N=146) population: current effective population size (N_0; top left), ancestral population size (N_1; top right) and the time (T) since the occurrence of the demographic change (below). The four runs are presented and differentiated by the type of line, as reported by the inherent subtitle. Produced in RStudio v. 1.4.1106.

4 Discussion

4.1 Contextualization of genetic diversity within and between species and populations

The first question in this research was about the expectation of finding high levels of genetic diversity in both populations of colobus monkeys, as a direct result of the cohesive forest habitat they inhabit. The results from the complete databases (BWC with 25 individuals and 15 loci, and RC with 146 individuals and 14 loci) will be discussed here, as the differences in genetic diversity in the databases with less loci are negligible. As expected, a relatively high genetic diversity was found in the genetic data of the two GRNP's colobus populations. The total number of alleles and the number of effective alleles was higher in RC than in BWC; however, the difference between Na and Ne was bigger in RC than in BWC. Therefore, in the case of the number of effective alleles, we can assume that the red colobus population is genetically more diverse than black-and-white colobus population, as the measure is less sensitive to sample sizes and rare alleles. Even though heterozygosity was high in both species, higher values of heterozygosity were found in RC and a bigger difference between expected and observed heterozygosity was found in BWC. This result is comprehensible considering that this species

also presented a higher value of the inbreeding coefficient (F_{is}), which can be a consequence of the homozygosity excess and null alleles being more frequently detected in BWC, as indicated by results from the program MICROCHECKER v. 2.2.3 (Van Oosterhout et al., 2004). A positive F_{is} may be the result of inbreeding or nonrandom mating in the population, which may happen in the context of a limited dispersal of individuals between populations or sections of the forest (Di Fiore, 2003). Overall, these results suggest that the RC population is more genetically diverse and has less inbreeding that the sympatric BWC population. However, comparisons between the two cases are problematic, as the number of samples in each population differs greatly (among other species- and population-specific characteristics) and they are different taxa with independent evolutionary trajectories and adaptation strategies. On the other hand, some measures of genetic diversity such as allelic richness provide a more unbiased observation of diversity, considering that it is not so sensitive to sample size. The results from this measure are congruent with the other values, presenting the RC population as having more allelic richness than that of the BWC population. Still, it would have been useful to apply a non-linear regression model such as the one described by Bashalkhanov et al. in 2009 to confirm that the small sample size in the black-and-white colobus population was not biasing the results of genetic diversity. Another possibility would be to statistically account for the disparity of the sample numbers between the *C. polykomos* and *P. b. badius* populations with rarefaction on private alleles (Kalinowski, 2005). In order to contextualize these results, another table was prepared (Table 4.1) with N_a, H_e, H_o and A_r from the populations in GRNP with the same parameters of the same species in Cantanhez National Park (CNP), Guinea-Bissau, (Minhós et al., 2013a) and Taï National Park (TNP), Ivory Coast (Minhós et al., 2023). All three studies used the same microsatellite markers to compare the same species across different West African protected areas. The subspecies of red colobus present in CNP is different from the one in GRNP and TNP, being the most western subspecies, the Temminck's red colobus (*P. b. temminckii*). Overall, the parameters from the CNP populations were the lowest and the ones from TNP were the highest (Table 4.1). Therefore, we can situate the case of GRNP between the other two locations, but closer to the TNP's case. The comparison with the other two studied protected areas suggests that the overall genetic diversity of colobines in the GRNP is high, especially considering the smaller size of the study area compared with the TNP, which has the highest values of genetic diversity. It also makes sense that the results from CNP had the lowest parameters of genetic diversity, considering that its forests are highly fragmented and contain human settlements within. I cannot however discard that the evolutionary history of the different red colobus subspecies may contribute to their different levels of genetic diversity. However, the fact that both *C. polykomos* and *P. badius* show the same trend in terms of genetic diversity in the different West African protected areas suggest that the differences found may be, at least partially, explained by the contrasting levels forest fragmentation. Thus, the comparison of the genetic diversity levels of the different protected forests suggests the importance of maintaining large, cohesive and relatively undisturbed forest blocks in order to preserve these colobines.

Table 4.1 Comparative table of the genetic diversity of *C. polykomos* (BWC) and *P. badius (*RC) populations from Cantanhez National Park, Gola Rainforest National Park and Ta National Park. Subspecies of red colobus in TNP and GRNP is *P. badius badius*, while in CNP the subspecies is *P. badius temminckii.* Data from the present study are in bold.

C. polykomos	Samples	N_a	H_o	H_e	A_r	F_{is}
Cantanhez N. P	52	4.5	0.475	0.415	2.059	- 0.138
Gola Rainforest N. P.	**25**	**5.267**	**0.523**	**0.645**	**4.943**	**0.219**
Taï N. P.	8	5.455	0.692	0.791	5.076	0.135
P. badius	Samples	N_a	H_o	H_e	A_r	F_{is}
Cantanhez N. P	**72**	4.8	0.538	0.508	1.634	- 0.045
Gola Rainforest N. P.	**146**	**8.857**	**0.668**	**0.716**	**8.269**	**0.077**
Taï N. P.	29	10.100	0.786	0.808	9.958	0.027

4.2 Overall absence of sharp population substructure

The first STRUCTURE analyses (Pritchard et al., 2000) conducted on the BWC population found two differentiated genetic clusters (Figure 3.7 and Figure 3.8) that were then explained by the presence of a group of highly related individuals (Figure 3.9 and Figure 3.10). After their removal, I found no genetic structure for the BWC population, a result that was confirmed by the PCA and by the analyses of the Mantel test and IBD pattern (Figure 3.20, Figure 3.22, Figure 3.23, respectively). Altogether, these results suggest that the BWC population has not been experiencing constraints in moving across the GRNP landscape, with no major barriers to their dispersal identified. Moreover, for this colobine population, Euclidian geographic distance seems to play the main role explaining the distance between individuals, as evidenced by the detection of an Isolation-by-distance pattern.

Graphical results from STRUCTURE for the RC population display an admixed population, where individuals do not clearly belong completely to one cluster or the other (Figure 3.11 and Figure 3.12). The results from the ΔK and L(K) calculation, as well as the posterior probability identifies the most likely number of clusters at K=2 (Figure 3.18, Figure 3.17, and Table 3.10, respectively). However, one must take care when using the ΔK to infer the correct number of genetic groups, as it has a bias towards K=2 (Cullingham et al., 2020). As for the K=2 result after the calculation of posterior probability, this finding may be explained by the presence of null alleles that were also detected in the population – since they can lead to an overestimation of K (Pritchard et al., 2010). The latter option is possibly the best explanation in this case, because after repeating the same analyses (Annex 7.4, Table 7.6) for the population without two out of three of the loci with errors such as null alleles, the posterior probability calculation gave K=1 as the most likely solution. The PCA further supports one cohesive population without substructure, with only two individuals that were not included in the inertia ellipse. However, the disruption of the IBD pattern, suggests that the distribution of the genetic diversity in this population is not fully explained by the Euclidean geographical distance. It could have been interesting to include here, for example, an RDA analysis – a combination of PCA and multiple regression – to further illuminate the patterns of distribution. Considering the geographic locations of the samples in the next analyses will be beneficial to illuminate the pattern of substructure within this population, as we will see below.

Nevertheless, there is a possibility that the impact of disturbance on both populations is still too recent to leave its genetic signature (Hoffman et al., 2017). Considering that these colobines have been historically hunted for subsistence (Linder et al., 2021), the animals living in the most accessible parts of the forest may be hunted. This has been shown to occur in GRNP, with colobines being more affected by this pressure than other primates (Bulte et al., 2013; Davies et al., 2008). For instance, a recent study estimated a nuclear density of 0.54 hunting signs per kilometer and 0.86 shots per day from firearms in the park (Foglietti, 2020). This may result in a localized avoidance of dispersal in some areas where hunters have more access, creating an artificial barrier to the movement of individuals. The past logging concessions in the park, especially in Gola South and western zone of Gola Central, could also have this kind of impact on genetic connectivity. Commercial logging lasted until 1989 and a logging moratorium was approved by the government in 2004 (for more details, see Klop et al., 2008). The absence of clear genetic structure on the two colobine populations suggest that the rivers and roads do not appear to be a serious obstacle for their movement. This is due to the fact that, when the genetic clustering is applied to the map, the few divisions that exist do not overlap on any of these landscape obstacles. Even the samples from Tiwai Island form a cohesive genetic structure with the ones from mainland Gola forests. The Moa River (Figure 4.1) becomes smaller during the dry season and colobines are able to cross it (Catherine Hill, person. communication), which makes genetic flow across the landscape possible. This is in agreement with what was found for both species in the similar context of the Taï National Park

(TNP) (Minhós et al., 2023), where the cohesive forests still seem to provide an ideal habitat for the colobines despite raising threats.

There are many factors that can affect dispersal of individuals throughout their range, shaping substructure within and between populations. These can result from local barriers, heterogeneous landscape mosaics and/or gradients (Balkenhol et al., 2014), gene flow and drift (Di Fiore, 2003), historical events (Colyn et al., 1991) and even species-specific biology and ecology (Basto et al., 2016; Radespiel & Bruford, 2014). That is one of the main reasons for the difficulty associated with the task of selecting the most probable number of genetic clusters in a population (Meirmans, 2015). Additionally, estimates of the optimal number of K have a pertinent degree of uncertainty (Evanno et al., 2005), while IBD patterns can be spurious and result from several different processes (Meirmans, 2012). That is the reasoning behind further analyses that take into account the geographic locations of the sampled individuals (Meirmans, 2015), as the patterns of substructure in both populations can become clearer and more unbiased. For example, in a genetic study of cougars (*Puma concolor*) using microsatellite loci, researchers illustrated a complex hierarchical genetic structure of a population in Idaho, USA (Balkenhol et al., 2014). The combined use of clustering methods and individual-based genetic distances may enable researchers to understand the interplay between heterogeneous landscape features and geographic distances shaping the animals' genetic structure across their area of distribution. Here, considering the geographic locations of the samples in the next analyses will further elucidate on the pattern of substructure within this population.

4.3 Spatial genetic analysis suggests genetic clusters on the study area

Referring to the spatial autocorrelation analysis, the correlograms of the BWC population indicated significantly related individuals at the closest ranges and significantly less relatedness at greater distances. As expected, these results are consonant with the IBD pattern and with the group of highly related individuals that was found in this population. Although the number of possible clusters in the population (Kmax) value was high, the admixture proportions and structure tessellation revealed three clusters in this population: two main ones and another one between them in the middle of the Gola South block (Figure 3.33). The latter has been confirmed as the cluster produced by the group of highly related individuals (yellow cluster on the referred map), as indicated by its absence in the results of the dataset without their presence. Without this cluster, the population still subdivides in two clusters (blue and green zones on the map below (Figure 4.1), suggesting that this population does have some degree of substructure, which is a common finding in this kind of analysis. For example, genetic diversity and population structure in the white-headed langur (*Trachypithecus leucocephalus*) was investigated across its main distribution area (Wang et al., 2017). While the population was divided into two clusters by the non-spatial structure analysis, the spatial one yielded more information about the effect of habitat fragmentation in the population, which coincided with known anthropogenic barriers to dispersal. The results from the primates' genetic data informed researchers of two management units of the species, thus aiding directly in plans for its conservation.

However, in this case it was not possible to identify a physical barrier to dispersal of black-and-white colobus in Gola South. There is a river and a road on the left of the cluster division (Figure 4.1), which do not seem to explain the barrier to the dispersal of individuals. The Mahoi river is the closest possible barrier to the cluster division seen on the map. Still, it is probably not a barrier to dispersal of these arboreal primates, as the forest canopy seems to be a sufficiently connected for the individuals to transpose the river (Isa Aleixo-Pais, personal communication).

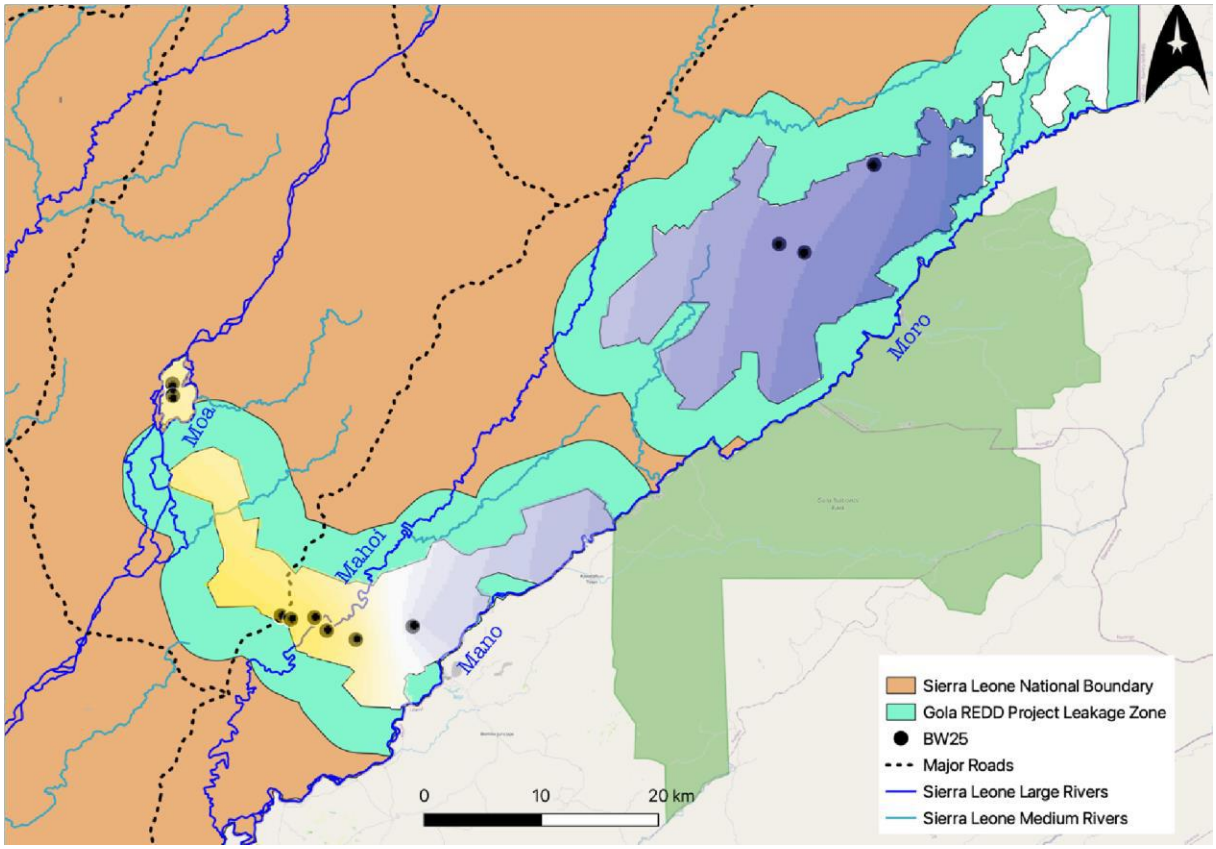


Figure 4.1 Superposition of cluster map from the tessellation of the BWC population on the study area map. With this illustration, it is possible to illuminate potential barriers to the dispersal of colobines. Prepared using TESS v. 2.3.1., CLUMPP v 1.1.2., RStudio v. 1.4.1106., QuantumGIS v. 3.16.10-Hannover.

In this case, there may be a historical and anthropogenic barrier that created this genetic differentiation. Since the Gola South block was commercially explored for timber some decades ago (logging operations finished in 1989) (Davies, 1987), the isolation between clusters could simply be the product of the time when they had more difficulties dispersing through the logged parts of the Gola forests. However, this species does have a history of tolerance to changes in habitat (at least to a certain degree), adapting through behavioral changes (Chapman et al., 2000; Minhós et al., 2016). Thus, hunting pressure – as indicated by Davies (1987) – may have driven the animals away from the logged and consequently more hunter-accessible forests of Gola South. Either way, genetic signals left by barriers to dispersal can take a few generations to appear in the animals' genetic signature, thus becoming detectable some decades later (Tung et al., 2010). In this case, the results do not seem to indicate a clear barrier for the dispersal of the animals, and the substructure is partially explained by a set of highly related individuals, with the rest of the population not exhibiting a strong differentiation.

Another possible explanation could come from the probabilities of finding more related individuals in the BWC population than in the RC population. The sampling scheme in this study results in a pattern similar to the typical research sampling simulated by Schwartz & McKelvey in 2009 (although technically the sampling scheme used here is linear). The authors demonstrated how one population that presents neighbor mating can form local patterns of relatedness, which coincided with the sampling areas. Thus, the association between high localized relatedness (which is what was found in this population, along with a significant degree of inbreeding) may indeed coincide with the location of groups of samples, thus creating spurious clusters. This would hardly happen in the sympatric red colobus population because even though both species have the same ecological pressures, red colobus groups are usually considerably bigger than black-and-white's (maximum 60 animals in the former and 16 in the latter) and the within group relatedness seems to be more easily diluted. Thus, the chances of coincidently finding more samples of related individuals in the BWC population would be, in theory,

higher than in the RC population. Furthermore, Klop et al. (2008) found several individuals of black-and-white colobus in the Gola North block in their survey, a block that was not examined in the context of this study. Therefore, the inclusion of samples from Gola North and more samples from Gola Central in a landscape genetic analysis could contribute to the clarification of the factors affecting the distribution of the genetic diversity in this population.

In the RC population, the spatial autocorrelation pattern revealed significantly more related dyads at the closest distances (up to 0.5 km) at all scales. Additionally, a lower degree of relatedness than expected at random was found at around 3 to 5 km and from 25 to 27 km distances. This result is in concordance with the disruption of the IBD pattern, which may have been the result of these relatedness patterns. Males of the population presented similar patterns to the whole population, with high relatedness at the closest distances and lower at further distances, a pattern expected for the usually more philopatric sex. As for the females, they seem highly related at the social group level, but the correlograms also show they have slightly lower relatedness than expected except for immediately adjacent (3 km) and farthest (57 km) social groups. This would imply that they seem to avoid the closest groups and do not travel as much to the farthest regions from their home group. These results suggest that females are the sex more responsible for the genetic flow of individuals through the distribution area. They also indicate that females do not disperse as much to the immediately adjacent social groups, which may have close relatives. Additionally, the autocorrelation analyses confirm the structure results showing no strong substructure.

The fact that the RC does not exhibit substructure while it is more sensitive to habitat disturbance than the sympatric black-and-white colobus (Minhós et al., 2016) further reinforces the quality of their shared habitat. Since these two primate species are found in sympatry in the Gola forests, one would expect the same ecological pressures – and their superior impact on the red colobus (*P. b. temminckii*), as found in Cantanhez National Park (Minhós et al., 2016). Besides being more sensitive to habitat changes, the RC also seems to have a higher hunting pressure in the GRNP, as reported recently by Davies et al., in 2008. Since the RC population did not exhibit structure, the factors behind these trends in the BWC population in Gola are not so clear and deserve further scrutiny. The difference in the number of samples between the two species (25 for BWC and 146 for RC) represents the possibility of bias in the analyses for the BWC population. Therefore, it would be advisable to obtain a bigger number of samples in future studies (preferably in the overall Gola Landscape) and explore further the genetic flow and relatedness in both colobus species. Additionally, it could be beneficial to observe the distribution patterns with resistance maps in the program Circuitscape (Shah & McRae, 2008), which would include more information on what variables could be behind this slight substructure.

4.4 Dispersal is not significantly sex-biased

Even though I did not find a sex-biased dispersal pattern for any of the two species, but there are some tendencies that are worth discerning attentively. Both in the overall population and blocks, usually males of the BWC population had negative $m\text{AI}_c$, while females had positive values – except for the Gola Central block and the Transect 44, where the trend inverted. The overall trend makes sense in the species-specific context, considering that immigrant individuals are expected to have a negative $m\text{AI}_c$ score and more philopatric individuals should have a positive $m\text{AI}_c$ score. This is in concordance with the dispersal trends observed in another population of this species in Ta National Park (Korstjens et al., 2005), where observational data were collected and confirmed usual male dispersal. As for the inversion of the trend in a specific transect, it may be due to occasional dispersal of females to neighboring groups, which has also been recorded in the latter study, among others (Sterck et al., 2002, Minhós et al. 2013b). When both sexes disperse in this species, males might be traveling longer distances, while females may tend to disperse preferentially to neighboring groups (Harris et al., 2009; Minhós et al., 2013a). The authors came to this conclusion based on their findings in the comparison of pairwise relatedness within and between social groups of BWC (Minhós et al., 2013a) and of another species of black-and-white

colobus, *C. guereza* (Harris et al., 2009). They found higher relatedness between female dyads within social groups, while the average relatedness of males was lower within groups, in concordance with what is expected of Cercopithecine primates (Di Fiore, 2003).

In the overall population of RC, females had a more negative mAI_c score than males, which is in agreement with expectations, since females have been frequently seen as the sex more responsible for dispersal in this species (Minhós et al., 2013a; Struhsaker, 2010). This is also a result in line with the patterns of relatedness in the spatial autocorrelation analysis, where all individuals (but especially males) were significantly more related at the shortest geographical distances. These dispersal patterns were also found in the Tana River red colobus (*Piliocolobus rufomitratus*) in Kibale National Park (Miyamoto et al., 2013). In that context, the females (but not males) belonging to smaller groups exhibited a higher level of relatedness, which led the researchers to suggest that they could be restraining dispersal out of the natal group due to decreased intra-group competition. Thus, Miyamoto et al., 2013 hypothesized that the dispersal system in red colobus may be linked with scramble competition and group size, because females showed increased within-group competition in bigger groups, which favored dispersal rates and eventually lower relatedness among females. Although more examples of this connection are needed, it is a plausible explanation to the complex patterns of dispersal that red colobus species exhibit. It can also be the reason behind the bias at the block scale, which revealed an even more complex pattern of dispersal, although with a tendency in line with the whole population. At the finest scale of transects, the groups revealed more positive mAI_c values in females than in males. If this pattern is considered representative of reality, it could mean that either the species is presenting some male dispersal at the neighborhood level and/or that females of the population have some limitations to dispersal at the local level. The first option is possible, as occasional male dispersal (either voluntary or by expulsion) has been observed in populations of *P. tephrosceles* (Struhsaker, 2010), *P. rufomitratus*, *P. temminckii* and male-biased dispersal has been recorded in a *P. kirkii* subpopulation (Starin, 1991; Marsh, 1979; Siex, 2003; respectively, as cited in Struhsaker, 2010). As for limitations in dispersal, encounter rates of these animals in GRNP may support this, as they tended to be found more towards the Tiwai island and continue to the north of the Gola South block, and then only at the easternmost zone of the same block and continuing to the Gola Central and Gola North blocks (Klop et al., 2008). The samples of these animals that were collected for this study also follow this pattern, with only a couple of samples having been found in the center of the Gola South block (Figure 2.2).

Regardless of possible explanations for the movement of the animals, in both cases the results are not significant – possibly due to methodological limitations. Firstly, it was not possible to distinguish samples from adult (post-dispersal) individuals from juveniles and neonates (pre-dispersal). Consequently, the presence of individuals that have not dispersed yet may have masked the sexual bias in the dispersal of adults. Secondly, the mAI_c method used here has already been shown to detect bias only in situations where there is a strong signal present (Goudet et al., 2002). This means that if dispersal is sex-biased in the populations in study, the signal is too weak to be detected with the method used here. Furthermore, in both species more samples of females were collected than of males, which can also conceal the dispersal patterns (Goudet et al., 2002). The spatial genetic analyses might further reveal the patterns of relatedness and structure that could explain these trends of dispersal. In any case, more detailed observational studies could yield more information on the dispersal patterns of both species.

4.5 A history of demographic collapse

To understand the evolutionary history of these populations (which can also elucidate on their response to future scenarios), the demographic history of both species was investigated. It was found that both populations were historically large, but the expected stability was interrupted by bottlenecks for both species at different times. The BWC population presented a strong bottleneck signal which indicates a decrease of one order of magnitude from the ancestral effective population ranging from 7,787 to 8,397 individuals to a current size between 286 to 592 individuals. Interestingly, the current total numbers in

GRNP are estimated (from population density based on sightings in line transects) to be around 8,876 (Klop et al., 2008), a number close to the past effective population size calculated here. Furthermore, the recent demographic collapse seems to have happened between 1,741 and 3,284 years ago, at which point all species of *Colobus* had already diverged (Ting, 2008). Species of black-and-white colobus such as *Colobus guereza* possibly used riverine forests as refugia in the Last Glacial Maximum during the Pleistocene, later following the rivers as dispersal paths after the forests expanded (Reed & Bidner, 2004). Then came the Holocene, and after the warm and humid climate of the Atlantic period, the Subboreal chronozone brought drier and cooler climate conditions and the end of the African Humid Period (until 3,000 years before present) (Collins et al., 2017; Dupont & Schefuß, 2018). From 4,000 to 1,300 before present (BP) many forests in Atlantic Equatorial Africa (specifically in present-day Congo, Cameroon and Ghana) were replaced by woodlands, wooded grasslands, and grasslands, with signatures of these changes varying locally according to specific hydrological conditions (Vincens et al., 1999). Therefore, this constriction in the tropical forests during the Subboreal and Subatlantic phases of the Holocene and the consequent effects on organisms that depended on them may have had a detrimental impact on the colobus populations, including of BWC. Ultimately, it is possible that both colobus species probably have had their populations reduced due to the effects of past climatic change from the African Humid Period to a more arid climate. It is an interesting finding considering that colobine populations of CNP suffered a more recent bottleneck (probably due to local anthropogenic overharvesting of resources) (Minhós et al., 2016) and the signals of bottleneck for the populations of TNP were much more uncertain. For the red colobus the authors could not confirm the existence of a demographic change and for the black-and-white colobus, if there was a demographic change, it was more subtle and recent (over the last 200 years) (Minhós et al., 2023). However, it is important to consider a difference in methodology, as at the time of those analyses the generation time considered for these colobines was five years. At the time of this study, the generation time for the species was updated to ten years, which will provide differences in the estimate of the time at which the demographic change happened.

The RC population also presented a bottleneck signal, indicating a past effective population size between 14,131 to 15,551 individuals that fell to a current size between 2,794 to 6,151 individuals. Just like in the BWC population, the most recent estimate of the total current population of RC in Gola's forests (Klop et al., 2008) is close (14,831) to past effective population size estimated here. As for the time at which the demographic change possibly occurred, it was probably between 3,663 to 8,772 years before present, or in other words, spanning from the Atlantic and the Subboreal chronozones of the Holocene epoch (Wanner et al., 2008). By this time, all the species of *Piliocolobus* had diverged long ago (from the late Miocene to the Pliocene/early Pleistocene), so changes in their evolutionary history could not explain this demographic change (Ting, 2008). As such, the reasons contributing to this bottleneck could be related with bioclimatic changes and consequent modifications in the forest cover. The differences between the time at which the demographic contraction in the two species may have to do with their different resilience to habitat modifications and with the fact that the BWC is more resilient to the loss of forest habitat and can better persist in smaller forests (Gonedelé Bi et al., 2019; Minhós et al., 2013a). There is not much information on the vertebrate record in Western and Central Africa, as bone preservation tends to be poor and paleontological and archaeological research in the region is scarce (Steele, 2007). However, there is evidence indicating post-glacial fluvial refuges in the Pleistocene as important forces responsible for molding the subspecific radiation and population structure of colobines (Colyn et al., 1991; Reed & Bidner, 2004; Struhsaker, 1981). The early Holocene epoch (specifically the Atlantic phase) was characterized by wetter and warmer conditions in most of northern, equatorial, and south-eastern tropical Africa – where several primate species such as colobines lived. This period (designated as African Humid Period, spanning from ~11,5 to 5,5 thousand years ago) presented the ideal conditions for the expansion of forests but was interrupted at around 5,200/5,800 to 4,800 years BP by a sudden climatic exchange to arid conditions (Collins et al., 2017; Ivory & Russell, 2018). This abrupt change instigated the substitution of

forests for deciduous woodlands (Lézine, 2009), accompanied by an increased fire occurrence which further promoted desertification (Dupont & Schefuß, 2018). It is possible that these conditions also fragmented the rainforests in West Africa, creating the Dahomey Gap – a savanna corridor that covers present-day Ghana, Togo, and Benin (Salzmann & Hoelzmann, 2005). Arboreal primates such as the red colobus depend on the forested landscape to survive, so it would be expected that a decrease in forest area resulted in a decline of these primate communities (Reed & Bidner, 2004). If we factor in all these changes in the climate and biomes, we can understand why communities of RC could have had such a dramatic decrease in the effective population size at that point in time. Nevertheless, considering the deviation from convergence of the different simulations of demographic history performed for this population, we cannot exclude the possibility of a spurious bottleneck signal. This uncertainty will only be solved by running the demographic analyses of the RC dataset for a larger number of interactions, until convergence between runs is achieved.

4.6 GRNP's colobines in the West African context

The comparison of patterns of genetic diversity, population structure, dispersal, and demographic history of the two colobines between three protected areas can illustrate how the level of preservation of each territory contributes to the conservation of colobine populations. In light of the results presented in this study, it is clear that *C. polykomos* (BWC) and *P. b. badius* (RC) populations are well supported by the extensive and continuous forests of the GRNP, even though the protected area is not as vast as the Taï National Park (TNP), in the Ivory Coast. Both ecosystems present similar continuous forest formations that belong to the Guinean Forests Hotspot (IUCN, 2015) and provide essential habitat for the colobines, among many other wild species (Klop et al., 2008; McGraw & Zuberbühler, 2007). However, in the GRNP human settlements are situated in the outskirts of the protected area, in buffer zones containing community forests, while in the TNP there are some inhabitants in the protected area and many in the buffer zone. In both protected areas the two colobus species are illegally hunted, with these species being the most hunted in the TNP (Refisch & Koné, 2005). In comparison, both species of colobus present a great amount of genetic diversity, considering the extension of their habitat. Still, some amount of inbreeding was detected in *C. polykomos* in GRNP, probably linked to its current effective population size, which deserves an investigation as to ascertain the reasons for this non-random mating. As for the *P. b. badius* population, it presented optimistic results in terms of genetic diversity and structure, even though the species is a preferential prey for both human hunters (Davies et al., 2008) and chimpanzees (Teelen, 2008). The unexpected result of spatial substructure in the *C. polykomos* population but not in the *P. b. badius* population (the more sensitive colobus to environmental degradation) deserves further exploration. One can say that the populations of the GRNP have similar genetic status to the colobinesin TNP, even though there was no clear signal of a past bottleneck like in the populations studied there (Minhós et al., 2023). Furthermore, the substructure in the BWC population of the GRNP was found only when using landscape genetic tools, which have not been applied to TNP case.

This is a contrasting result to the genetic studies conducted with *P. b. temminckii* and *C. polykomos* in the fragmented forests of CNP, situated in Guinea-Bissau (Minhós et al., 2013a; Minhós et al., 2016), where spatial genetic analyses detected a possible disruption in the red colobus but not in the black-and-white colobus movement across the heterogeneous landscape. There, human settlements and roads develop throughout the protected area in a mosaic of forest and agricultural land, where deforestation has decreased the natural habitat. Although in all three protected areas colobus monkeys are hunted for bushmeat consumption (Minhós et al., 2023; Minhós et al., 2013b), the genetic studies indicate that the primates at CNP are more impacted by the human disturbance in their natural habitats. As for the lack of clear signs of the impact of past logging and present hunting in the GRNP's colobines, it is possible to explain in two ways: either the pressure is sustainable and is not severely affecting this population, or the pressures are so recent that the genetic signal is not yet detectable (Hoffman et al., 2017). For this reason, it is important to take these results with caution. Due to the limitation that the use of few genetic

markers has to unravel genetic patterns resultant from recent impacts, I cannot discard the possibility that the colobine monkeys from GRNP are already being impacted by hunting or habitat degradation in some areas of the park, but the genetic data used in this study lacks the power to detect it. It is therefore important to continue to monitor the genetic status of these different populations, ideally with application of non-invasive genomic approaches, as well as to continue the conservation efforts in the park (Linder et al., 2021). It seems that, for now, the two colobus species in the GRNP have expected levels of diversity and present no (red colobus) to little (black-and-white colobus) genetic substructuring with geographic data included.

The populations of colobus monkeys that live in more cohesive forests such as in the GRNP and TNP seem to have the healthiest populations in comparison to the CNP case, emphasizing the importance of preserving continuous tracts of protected forests to arboreal primates. This characteristic seems to be more important than the extension of the protected areas, as exemplified by the case of the Tana River mangabey (*Cercocebus galeritus*) that lives in a gallery forest of only 26 km^2 (Mbora & McPeek, 2015). The small but continuous tract of forest seems to be an important factor to provide the populations with sufficient food, space for dispersal and protection to maintain their genetic diversity. Considering this example, the levels of genetic diversity and population connectivity in the cohesive forests of the TNP and the GRNP are not surprising. Although the connection between genetic diversity and absence of deforestation is not always simple (Mitani et al., 2000), the results from this study presented in the context of similar cases in West Africa indicate the importance of continuous forests for these forest dwelling primates.

5 Concluding remarks

5.1 Limitations and possibilities

While efforts have been made to control data quality and to reduce the amount of bias in the results through repetitions and different methodologies, this study still has limitations. The limitation that could have the most serious impact in the analyses is the number of samples from the black-and-white colobus (Chikhi et al., 2010; Radespiel & Bruford, 2014). It is a number situated exactly at the acceptable limit for a genetic study using microsatellites (Hale et al., 2012), being far from the number of samples for the sympatric red colobus – which introduces difficulties to the comparison between the two species. The sampling scheme may also bias our results of landscape genetics analyses specifically with this species, since their group numbers are usually smaller. However, this limitation does not have many options to be surpassed besides trying to extend the fieldwork time to try to get more samples from each species in the Gola South block, which is already known for its lack of groups compared to the other blocks. Furthermore, the inclusion of samples from the Gola North block would be extremely interesting, since superior numbers of both colobus monkeys have been reported there (Davies, 1987; Klop et al., 2008).

As for possible improvements in the future, it would have been useful to analyze mitochondrial DNA in the context of this study, beside microsatellites. Being maternally inherited and subjected to lower mutation rates, this inclusion of mtDNA could have provided more information on the dispersal of individuals, which could possibly illuminate the origin of the patterns of movement of the black-and-white colobus that gave rise to inbreeding and geography-linked substructure. Furthermore, this additional analysis could give more detail to the complex dispersal patterns that were found in the red colobus population. Finally, microsatellites and conservation genetics have been superseded by genomic data (the complete set of genetic information present in an organism, including mitochondrial, nuclear and chloroplastic – in the case of plants – material) and conservation genomics for some time (Salgado-Lynn et al., 2016). The use of this methodology for future studies of primate populations will provide more detail and help solve essential conservation genetics questions that have been difficult to answer until now due to the lack of power that few molecular markers have to infer recent and subtle patterns

(Allendorf et al., 2010). For example, next-generation sequencing techniques make possible the simultaneous sequencing of millions/billions of brief sequences that can be reassembled into a major segment of the genome of an organism. Single nucleotide polymorphisms, the most abundant and widespread polymorphic marker in a genome, are thought to be the future preferential genetic marker in ecology, evolution, and conservation studies of primates (Salgado-Lynn et al., 2016). The reduced amount of DNA present in non-invasive fecal samples has been a problem for genomic studies, but this field is advancing rapidly, and it keeps presenting new methodologies to study non-model wild endangered populations (Salgado-Lynn et al., 2016), such as the ones presented here. For instance, the team I'm working with is already analyzing other populations in Sierra Leone, using non-invasive genomic approaches. The data that will become available from that investigation will inform even further on the conservation status of these species at the national level. It may also situate more clearly their conservation status in comparison with other populations in different regions of West Africa.
In terms of improvements in the analyses themselves, beside the ones previously referred, it would be also beneficial to perform a population viability analysis (PVA) for these populations (e.g., with the software VORTEX (Frankham, 2017)). With this analysis, it is possible to estimate the probabilities of maintenance of these populations, thus helping in their conservation planning in the GRNP. Different variables and plans can be simulated in this way, before deciding on the best courses of action for this specific context. Ideally, populations of other areas in Sierra Leone (protected or not) should also be assessed and included in this analysis – not only for the PVA but for the same analyses that the populations presented here were submitted to. In other words, a country wide assessment would be great to contextualize these populations in Sierra Leone. Eventually, this investigation should be extended to neighboring countries, in order to provide the full picture of the genetic status of these species.

5.2 Contribution to conservation of West African colobines

In this investigation, the findings are generally in concordance with the knowledge collected so far about African colobus monkeys. Here, contributions are added to the foremost priority of the recently published red colobus conservation plan (Linder et al., 2021) – the investigation on red colobus populations to inform their conservation planning. Even though 75% of red colobus species are threatened or critically threatened with extinction, only few populations have been genetically studied. The black-and-white colobus populations declined more than 50% in the last thirty years, as a consequence of the same pressures as the red colobus and many other wild, irreplaceable species (Gonedelé Bi et al., 2019). The populations of the colobus monkeys of the GRNP had not yet been the object of a genetic study, until now. These recent data provide an important contribution to knowledge on these two non-human primate species, which can be used in future conservation plans. For example, the Gola forests surpass the national boundaries of Sierra Leone and Liberia, and their conservation at a transboundary level has already initiated, with a view to protect the largest remaining block of the Upper Guinean Forests of West Africa (USAID/WA BiCC et al., 2020). One possible outcome that this partnership could have would be an international project with the objective of studying communities of different organisms at the whole Gola Landscape level. It could yield interesting results on the animals' movement within these forests and provide relevant data to better plan their conservation.
Although it is complicated to perceive clearly what the rates of land change and deforestation in Sierra Leone really entail (Wadsworth & Lebbie, 2019), we can observe nonetheless the trend of agricultural expansion and land conversion that leads to biodiversity loss (Government of Sierra Leone, 2017; Norris et al., 2010). The consequences to primate populations are serious, as exemplified by past (possible) extinctions (Oates et al., 2020). While the results presented here referring to the populations of colobus in the GRNP are optimistic, one must remember that they are so only in the light of their conservation status. Therefore, these protected populations are also an important safeguard for these colobus species both in Sierra Leone and Liberia, which share the Gola Landscape and high deforestation rates. If these populations are to be maintained at healthy levels, it is essential to conserve protected areas and to

safeguard the essential habitats, avoiding/reducing their fragmentation and eventual destruction (Fernández et al., 2022). It is also crucial to work with human populations that live beside these wild animals to aid in their conservation, taking their needs and cultural differences into account (Aleixo-Pais, 2022; Lee, 2010; Riley, 2006). Since most of global lands (including forests and farmlands) are owned or managed by smallholders, local communities, and Indigenous Peoples (FAO, 2022), they are the ones most impacted by conservation programs. Similarly, they can have a great impact on conservation by providing ecological knowledge and building long-term foundations for the sustainability of those projects (Estrada et al., 2022). Therefore, the local communities must be well integrated on their essential position as stewards of the natural heritage that benefits us all.

6 Bibliography

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7 Annex

7.1 Supplementary materials relating to quality analyses.

Table 7.1 GEMINI v1.3.0 software estimate of minimum number of PCR repetitions across loci for highest degree of confidence in the genotypes (relating to the BWC population).

N of repeats (threshold)	1(-)	2	3	4	5	6	7	8	9	10	11	12
% correct_id.	32.30	76.26	86.03	97.23	99.14	99.70	99.92	99.97	99.99	99.98	99.99	99.99
%wrong_id.	00.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
%new_id.	67.70	23.74	13.97	2.77	0.86	0.30	0.08	0.03	0.01	0.02	0.01	0.01

Table 7.2 GEMINI v1.3.0 software estimate of minimum number of PCR repetitions across loci for highest degree of confidence in the genotypes (relating to the RC population).

N of repeats (threshold)	1(-)	2	3	4	5	6	7	8	9	10	11	12
% correct_id.	59.44	85.48	93.39	99.05	99.63	99.73	99.96	99.98	99.99	100.00	100.00	100.00
%wrong_id.	00.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
%new_id.	40.56	14.52	6.61	0.95	0.37	0.27	0.04	0.02	0.01	0.00	0.00	0.00

7.2 Supplementary materials relating to the genetic diversity analyses

Table 7.3 Supplementary material relating to genetic diversity analyses in the BWC population with 12 loci and 25 individuals.

Locus	N	Na	Ne	Ho	He	Ar	HWE	Bonferroni	Fis
D13s321	23	6,00	4,182	0,609	0,761	5,615	0,160	NA	0,221
D2s136	25	7,00	3,181	0,480	0,686	6,242	0,012	0,05	0,318
D6s474	24	5,00	3,752	0,875	0,734	4,927	0,149	NA	-0,172
D10s611	25	3,00	1,863	0,480	0,463	2,997	0,900	NA	-0,016
D4s2408	22	6,00	3,237	0,500	0,691	5,833	0,000	0,003	0,298
D1s548	18	7,00	5,684	0,667	0,824	6,748	0,110	NA	0,218
D2s442	21	5,00	2,892	0,667	0,654	4,575	0,508	NA	0,005
D11s2002	20	7,00	5,000	0,800	0,800	6,613	0,673	NA	0,026
D12s372	20	5,00	1,831	0,350	0,454	4,799	0,238	NA	0,253
Fesps	24	4,00	2,612	0,500	0,617	3,611	0,136	NA	0,210
D6s1056	23	5,00	1,899	0,435	0,474	4,369	0,476	NA	0,104
D10s1432	25	5,00	3,213	0,760	0,689	4,820	0,532	NA	-0,083
Mean	**22,500**	**5,417**	**3,279**	**0,593**	**0,654**	**5,096**	**0,325**	**-**	**0,115**

7.2 Supplementary materials relating to the genetic diversity analyses

Table 7.4 Supplementary material relating to genetic diversity analyses in the RC population with 12 loci and 146 individuals

Locus	N	Na	Ne	Ho	He	Ar	HWE	Bonferroni	Fis
D13s321	141	8,000	3,064	0,617	0,674	7,631	0,305	NA	0,088
D2s1326	146	14,000	5,174	0,829	0,807	12,955	0,000	0,0036	-0,024
D6s474	142	15,000	5,889	0,810	0,830	14,004	0,000	0,0036	0,028
D4s2408	146	9,000	5,535	0,822	0,819	8,546	0,968	NA	0,000
D1s548	142	9,000	4,421	0,810	0,774	8,450	0,296	NA	-0,043
D11s2002	143	8,000	4,331	0,713	0,769	7,716	0,001	0,0036	0,076
D12s372	137	5,000	1,661	0,504	0,398	4,677	0,000	0,0036	-0,263
Fesps	135	8,000	4,991	0,800	0,800	7,735	0,585	NA	0,003
D1s1665	141	5,000	3,173	0,631	0,685	5,000	0,242	NA	0,082
D6s1056	145	7,000	3,016	0,759	0,668	6,715	0,225	NA	-0,132
D10s676	136	9,000	6,512	0,757	0,846	8,747	0,001	0,0036	0,109
D10s1432	145	10,000	4,659	0,779	0,785	9,582	0,000	0,0036	0,011
Average	**141,583**	**8,917**	**4,369**	**0,736**	**0,738**	**8,480**	**0,219**	**-**	**-0,005**

7.3 Supplementary materials referring to structure analyses

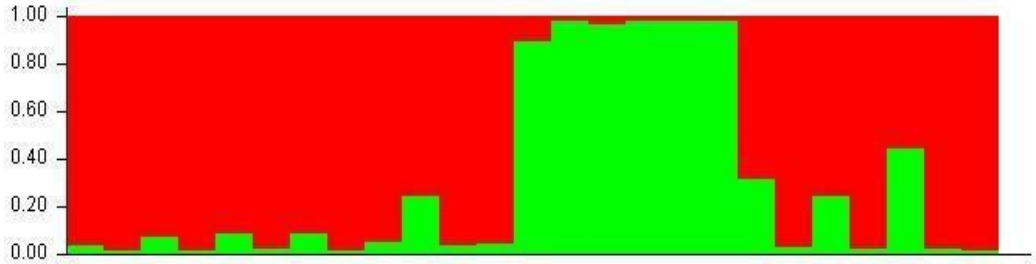

Figure 7.1 STRUCTURE analysis of BWC population with 25 individuals and 12 loci, with K=2.

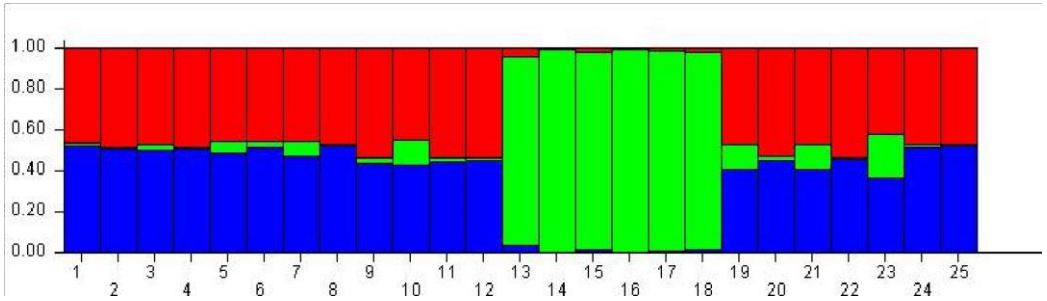

Figure 7.2 STRUCTURE analysis of BWC population with 25 individuals and 12 loci, with K=3.

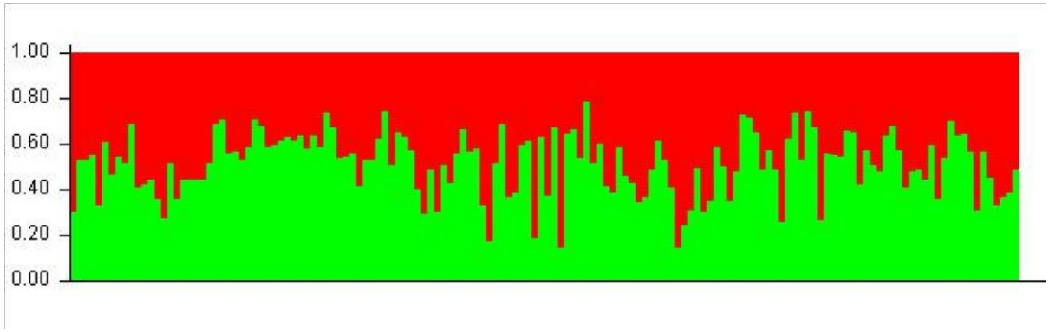

Figure 7.3 STRUCTURE analysis of RC population with 146 individuals and 12 loci, with K=2.

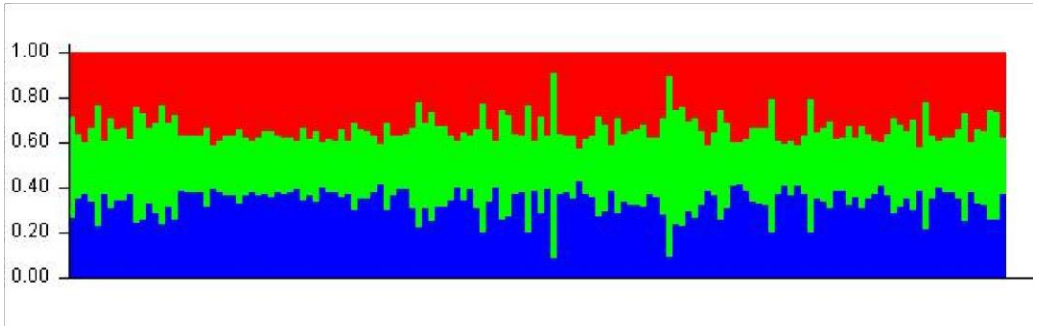

Figure 7.4 STRUCTURE analysis of RC population with 146 individuals and 12 loci, with K=3.

7.3 Supplementary materials referring to structure analyses

Table 7.5 Calculation of posterior probability of K for the BWC population with 25 individuals and 12 loci.

BW	12	1	2	3	4	5
K	Ln(P)	-739,90	-704,92	-752,08	-771,38	-803,82
1	-739,90	-1,00	-35,98	11,18	30,48	62,92
2	-704,92	33,98	-1,00	46,16	65,46	97,90
3	-752,08	-13,18	-48,16	-1,00	18,30	50,74
4	-771,38	-32,48	-67,46	-20,30	-1,00	31,44
5	-803,82	-64,92	-99,90	-52,74	-33,44	-1,00
Probability		**0,00**	**1,00**	**0,00**	**0,00**	**0,00**

Table 7.6 Calculation of posterior probability of K for the RC population with 146 individuals and 12 loci.

RC	12	1	2	3	4	5
K	Ln(P)	-5506,00	-5689,38	-5769,34	-5626,06	-5507,90
1	-5506,00	-1,00	182,38	262,34	119,06	0,90
2	-5689,38	-184,38	-1,00	78,96	-64,32	-182,48
3	-5769,34	-264,34	-80,96	-1,00	-144,28	-262,44
4	-5626,06	-121,06	62,32	142,28	-1,00	-119,16
5	-5507,90	-2,90	180,48	260,44	117,16	-1,00
Probability		**0,86989**	**0,00000**	**0,00000**	**0,00000**	**0,13011**

7.4 Supplementary materials referring to spatial structure analyses

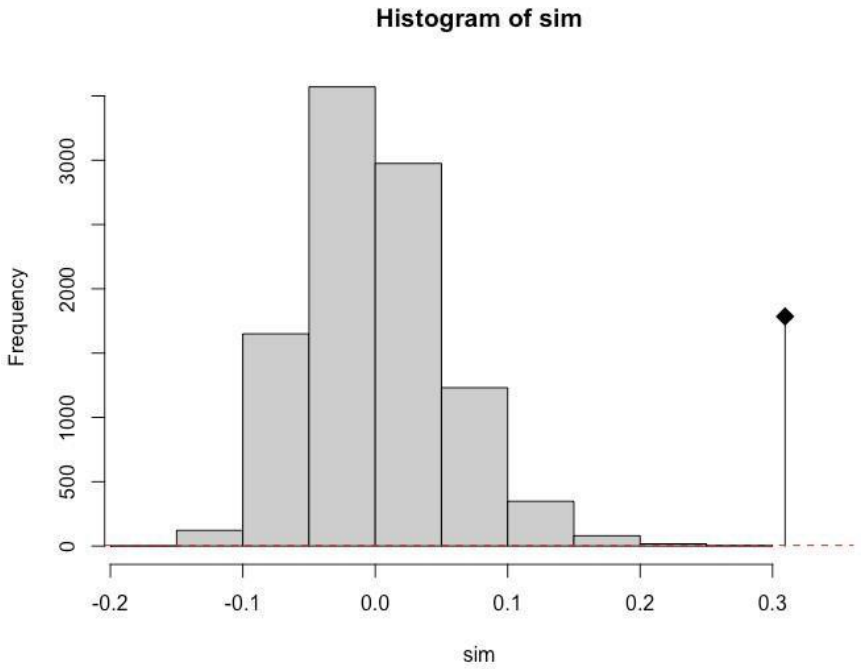

Figure 7.5 Mantel test of IBD for the BWC population with 25 individuals and 12 loci.

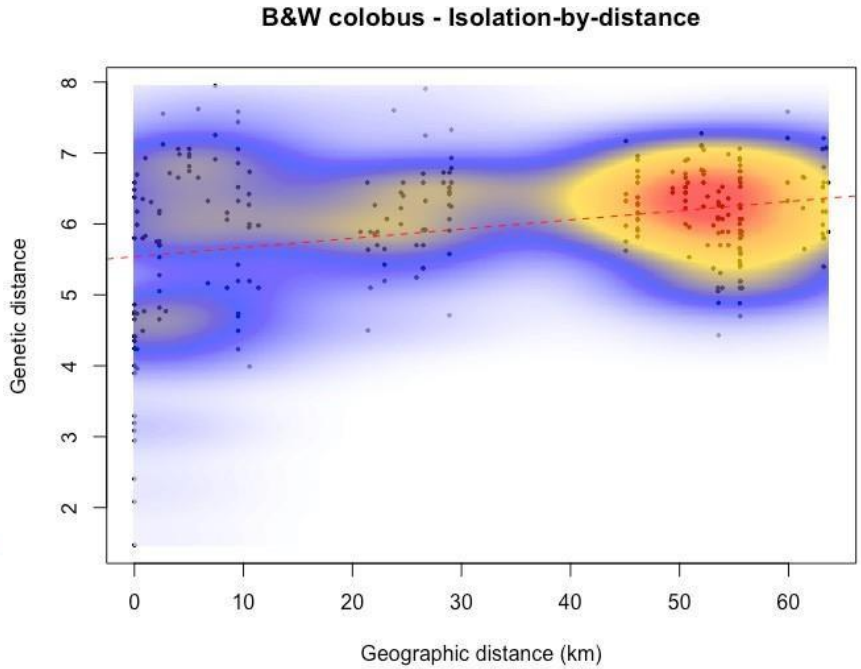

Figure 7.6 Scatterplot of isolation by distance pattern in the BWC population with 25 individuals and 12 loci.

7.4 Supplementary materials referring to spatial structure analyses

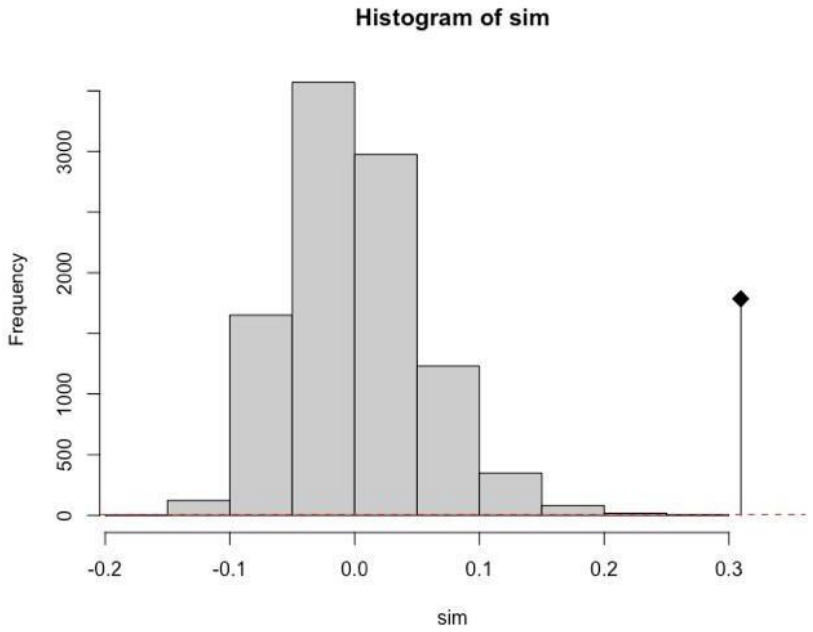

Figure 7.7 Mantel test of IBD for the BWC population with 25 individuals and 12 loci.

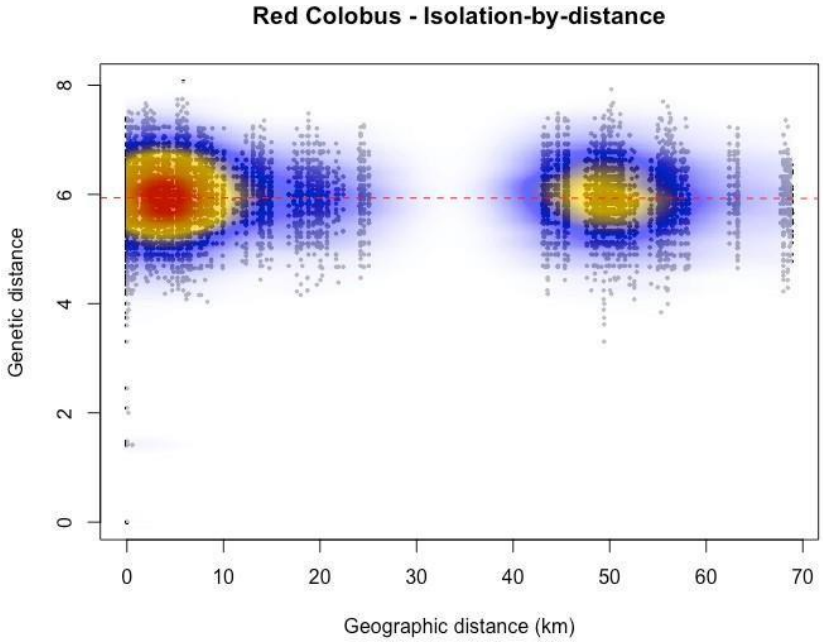

Figure 7.8 Scatterplot of isolation by distance pattern in RC population with 146 individuals. population with 146 individuals.

7.4 Supplementary materials referring to spatial structure analyses

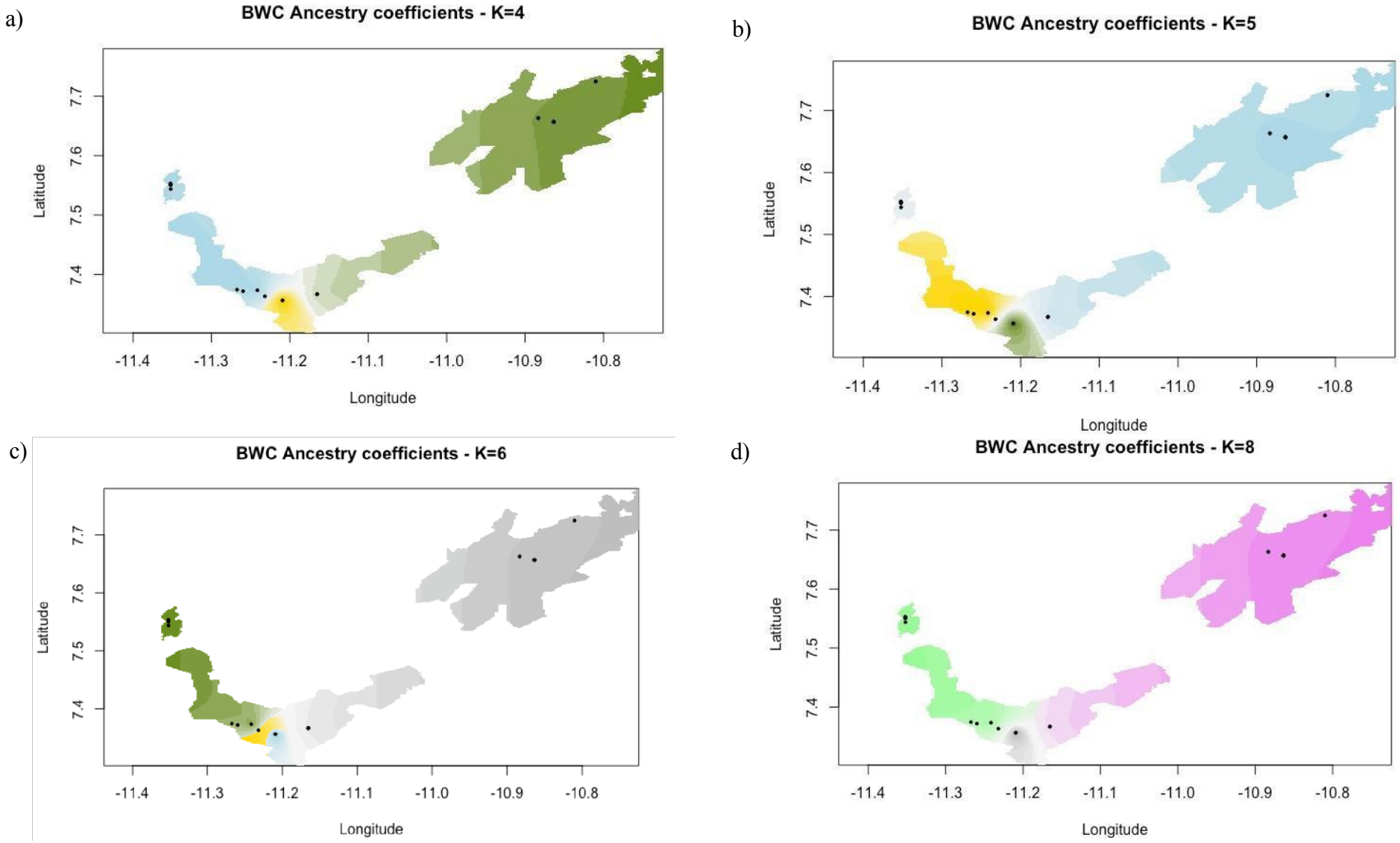

Figure 7.9 Supplementary figures of different clusters in the BWC population with 25 individuals and 15 loci. Structure tessellation of K=4 (a), K=5 (b), K=6 (c) and K=8 (d).

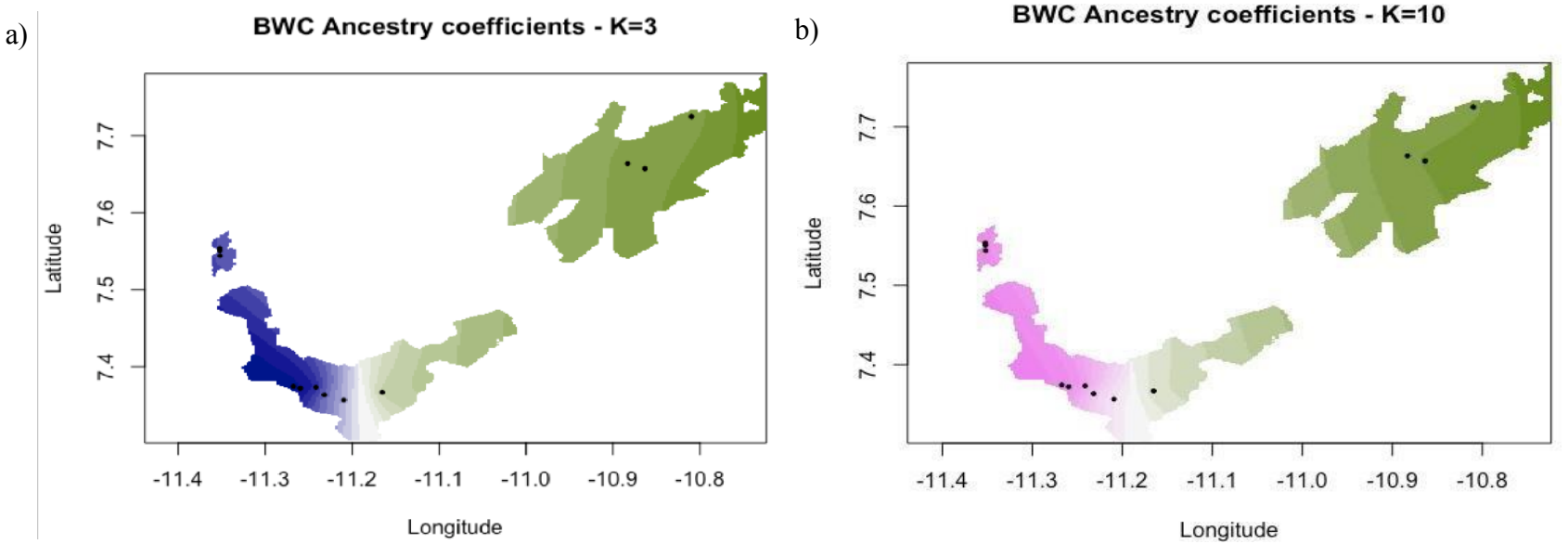

Figure 7.10 Supplementary figures of different clusters in the BWC population with 18 individuals and 14 loci. Structure tessellation of K=3 (a) and K=10 (b).

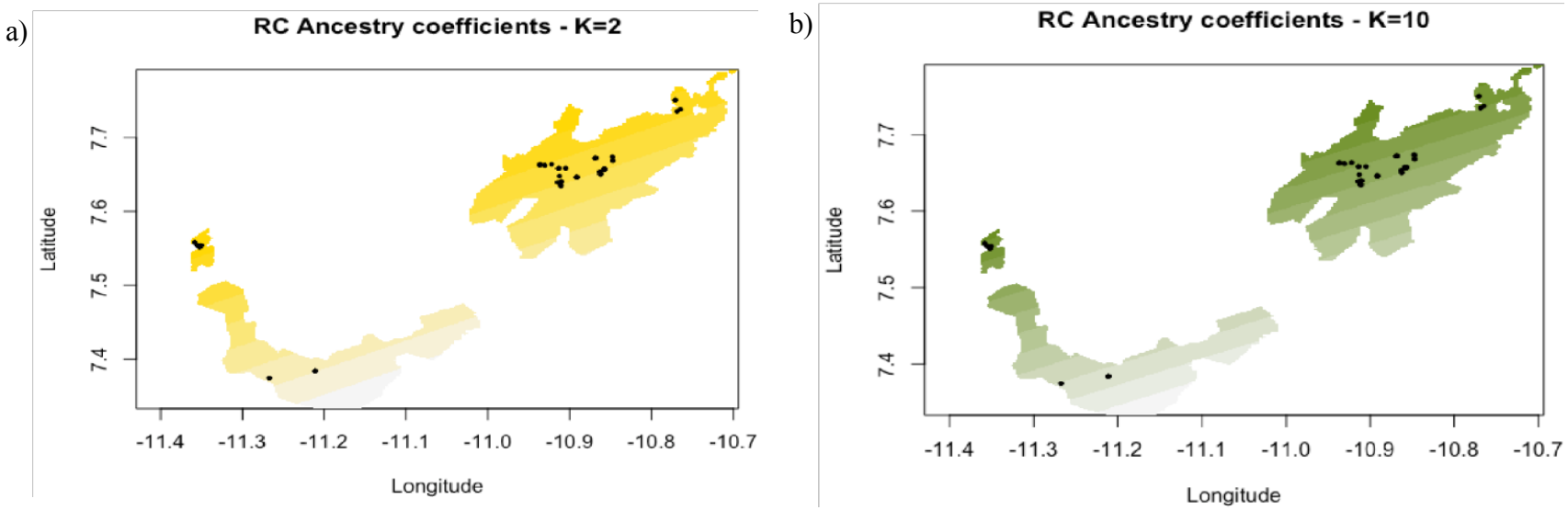

Figure 7.11 Supplementary figures of different clusters in the RC population with 146 individuals and 14 loci. Structure tessellation of K=2 (a) and K=10 (b).

7.5 Supplementary materials regarding demographic history analyses

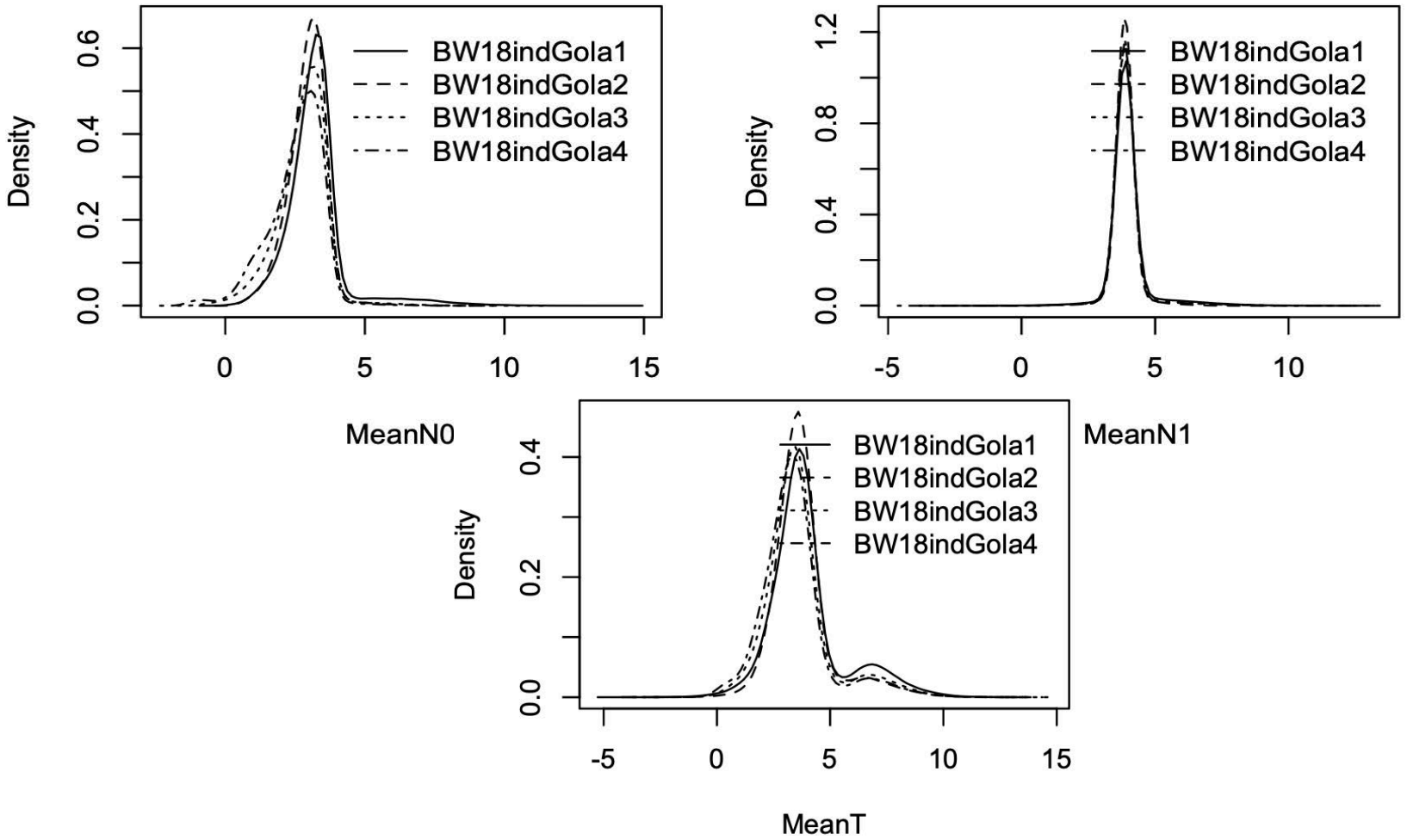

Figure 7.12 Posterior distributions of MSVAR 1.3 parameters' means in a logarithmic scale for the BWC population without related individuals (BWC with 18 individuals): current effective population size (N_0; top left), ancestral population size (N_1; top right) and the time (T) since the occurrence of the demographic change (below). The four runs are presented and differentiated by the type of line, as reported by the inherent subtitle. Produced in RStudio v.1.4.1106.