# ECOLOGY AND CONSERVATION GENETICS

# OF THE ENDANGERED

# MOUNTAIN BONGO

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# ECOLOGY AND CONSERVATION GENETICS OF THE ENDANGERED MOUNTAIN BONGO

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## General Abstract

Information on population status, distributions and genetics are paramount for effective species conservation. The mountain bongo (*Tragelaphus eurycerus isaaci*) is a critically endangered antelope endemic to the highlands of central Kenya. Although it has long been a focus of international conservation efforts, rigorous information regarding the status of wild populations are limited. This projects aims at delivering valuable information for managers and practitioners involved with the long-term conservation of this iconic antelope. The development of an individual identification system allowed the estimation of the size of remnant populations and their trend in recent years, for the first time. Furthermore, the implementation of the identification system allowed for a deeper monitoring and understanding of these populations, with estimates on sex and age specific vital rates. Results from population monitoring have informed the development of a new Species Distribution Model for bongo that relies on breeding populations to predict where, in the current range, areas suitable for breeding herds are located. Moreover, the current genetic diversity, inferred through mtDNA, of these two remnant populations have been assessed, while comparing this with that of the captive population found in extant matrilines of Europe, Kenya, and the US to elucidate on the risk of outbreeding depression in relying on the captive population for reintroduction. My results show an extremely low genetic diversity, with two haplotypes found in the wild, and only one of these represented in captivity. Moreover, I assess the influence that the relatedness of founders may have had on the current genetic situation of the captive population with the use of simulations applied to the bongo international studbook. Results indicate that the current low diversity found in captivity is likely due to an already depleted founder stock rather than a shortcoming of the captive breeding program. Findings presented here provide vital information for local authorities, managers, and practitioners involved with the development of actions and strategies for the long-term conservation of the critically endangered mountain bongo.

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The first people I need to thank are my supervisors, as I was lucky enough to have the opportunity to work under the supervision of not one but two: Ed Harris and Richard Preziosi. I began this journey in research with Ed Harris as my MSc supervisor, and I cannot thank him enough for his mentoring, support, and patience throughout all these years, I would not be who I am as a researcher and as a person without him. I feel extremely lucky to have had the opportunity to also work with Richard Preziosi, whose mentoring and constant support through the final stages of the project have helped me realise how much I have grown both as a scientist and as a person throughout my PhD. If I feel happy and confident in delivering this thesis is also thanks to him.

Martin Jones has been the first person I have met at MMU, and although I wish I had had more opportunities to work closely with him, he has always been available to provide support and advice on any issue I may have encountered, both academic and not. Bradley Cain is another person who has been an integral part of my research development throughout the years. I owe him so much for all of his insights, advice, and support in and out of the field and there is one aspect of what I consider my working ethos as a scientist that I fully owe to him: a profound respect for the local authorities. The understanding that I was working *for* Kenya rather than simply *in* Kenya is the one thing I will always carry with me in my career.

Thanks to the relevance given throughout the project to sharing and collaborating with local authorities, I feel like I have managed to make my work less about fulfilling my own aspirations and more about providing useful information for the preservation of the wonderful place I was working in, Kenya. For me, collaborating with the Kenya Wildlife Service has not been a bureaucratic necessity, but an important opportunity for me to grow and become a better researcher. KWS has provided logistical and security support throughout the project, and I therefore feel the need to thank all of the people in the Service that have helped and assisted me during this project. Fred Omengo of the Mountain Conservation Area research station has been the person I am most grateful to have encountered during my work with KWS; he has always been supportive, ready to provide assistance and useful advice and insights. I look forward to having the opportunity to work again with him in the future. The KWS scientists I had the opportunity to work with have provided assistance, support, and a good, and needed, dose of laughs during

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My interest in the Zoo community and in following a path that allows field conservation and zoos to be intertwined is largely owed to my experience at the Zoo where I used to work as an undergrad, and where indeed I grew up: Parco Natura Viva. Hence, I need to thank Katia Dell'Aira, my first employer, first supervisor and now a good friend, and Caterina Spiezio, who gave me the first insight into what it means to be a researcher co-supervising my undergrad project. Finally Cesare Avesani Zaborra, the director of Parco Natura Viva, for his support and for believing in me from the first time we met, it is thanks to him that I realised how much the Zoo community could, and should, be pivotal in field conservation.

As many other conservation biologists, I had dreamed of working in Africa since I was a little kid always glued to the television watching documentaries. I consider myself one of the luckiest persons on earth to have managed to realise this dream and it is only thanks to the support of some very special people, my parents Luca Sandri and Paola Gaspari. Since I was a little kid with a visceral passion for the natural world, my parents have supported me in order to allow me to always follow my passion, and try to make a living out of it. *Grazie Mamma*. *Grazie Papa'*. Moreover, if I took this path is also thanks to the constant support of my big brother, Nicoló Sandri, who has always seen in me more than I would ever see myself, I hope in these years I have managed to pay back some of this support. *Grazie Nico*.

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# Chapter 1:
# General Introduction

## Introduction

Preserving the natural world is an international priority (Brooks et al., 2006) with evidence suggesting that current extinction rates are comparable to those of past mass extinctions (Ceballos et al., 2015, 2017). While there are ethical imperatives to take actions to limit the loss of biodiversity (Ehrenfeld, 1976), there are also reasons to limit the impact that such loss would have on humanity due to altered ecosystems and the services they provide (Cardinale et al., 2012). Conservation biology (hereafter conservation) provides the framework for actions aimed at limiting the loss of biodiversity (Soulé, 1985).

Conservation actions can focus on different levels of the natural world. There exist initiatives aimed at the protection of entire ecosystems, where the main target of actions is at the landscape level, where by addressing issues and threats faced by entire ecosystems, conservationists can manage to limit biodiversity loss (Rodríguez et al., 2011). However, the usual targets of conservation have been species (Mace et al., 2008). Species-focussed conservation relies on actions on populations of a species of interest to ultimately help its recovery (Ceballos et al., 2017).

Effective conservation of populations requires information about demographics, distributions, and genetics, as these aspects are fundamental in the assessment of their status (Mace et al., 2008). Most research in species focussed conservation aims to elucidate questions regarding these aspects. This information can inform managers and practitioners, who then use it to design effective conservation actions. Knowledge of the vital rates and size of a population allows for the assessment of its viability, hence its extinction risk (Caughley, 1994; Lacy, 2019). Whereas, the understanding of habitat needs and distribution allows for a prioritisation of areas occupied by a species, or to inform where a population may be restored through reintroduction (Guisan et al., 2013). Moreover, information on the genetic makeup of populations can inform the need for actions aimed at augmenting genetic variation in small populations (Bell et al., 2019). Hence, this information provides the main foundations for any conservation action aimed at reversing the decline of a threatened species or population. By understanding the ecology and genetics of small and isolated populations, typical of threatened species, conservationists have provided effective solutions to limit the decline of species on the brink of extinction and even restore taxa already extinct in the wild (Sodhi et al., 2011).

Conservation actions, be it reintroductions or the institution of a protected area, are characterised by a decision process, where managers evaluate actions as needed or not depending on the available information (McCarthy and Possingham, 2007). It is therefore clear that any study aimed at gathering, refining, and augmenting current understanding is of relevance. While managers need reliable and extensive knowledge before undertaking actions, for critically endangered species actions should be designed and undertaken as soon as even basic information are available, in order to avoid  “counting the books while the library burns” (Lindenmayer et al. 2013). Moreover, even if actions were taken with limited data, the use of an adaptive management framework would allow novel information to be included, and subsequently adapt the actions accordingly (Williams, 2011; Serrouya et al., 2019).

The need for data to guide the development of effective conservation actions is of particular relevance when the target species is considered a flagship species, where its conservation can help in raising awareness and drive further conservation initiatives for an entire ecosystem (Caro, 2010); even more so when such flagship species can generate interest and awareness for an endangered ecosystem which provides essential services to local communities. That is the case for the mountain bongo (*Tragelaphus eurycerus isaaci*), an antelope endemic to the Afromontane forests of the central highlands of Kenya (Elkan and Smith, 2013).

## The Mountain Bongo

The mountain bongo (hereafter bongo) is the largest of forest dwelling antelopes, with males weighing up to 400 kg, and characterised by a striking coat pattern of 11 – 15 white stripes on a deep chestnut red coat (Figure 1). Bongo are also characterised by being highly social, with females living in herds with their young, whereas males are usually solitary and do not coerce females into harems (Estes, 1992). The mountain bongo is the only recognised subspecies aside from the nominate Lowland or Western bongo (*Tragelaphus eurycerus eurycerus*; see Gippoliti et al. (2018) for an alternative taxonomy) which inhabits the equatorial forest of Central and Western Africa (Figure 2; Elkan and Smith, 2013).

*Figure 1: An adult female mountain bongo. The image results from camera traps placed by the Author (Tommaso Sandri) as part of the project in the Aberdare national park.*

Bongo being an equatorial forest species, the mountain bongo (hereafter bongo) can be considered a marginal or relict population on the highlands of Eastern Africa. Such marginal populations are characteristic of other central African species, like the forest hog (*Hylochoerus meinertzhageni*) and the golden cat (*Caracal aurata*), and such a distribution likely results from the equatorial forest cyclical expansion throughout equatorial Africa during the Pleistocene (Kingdon, 2015). The retreating forest would persist solely in areas at higher elevations characterised by higher humidity, thus creating scattered forested islands across the landscape (Moreau, 1963).

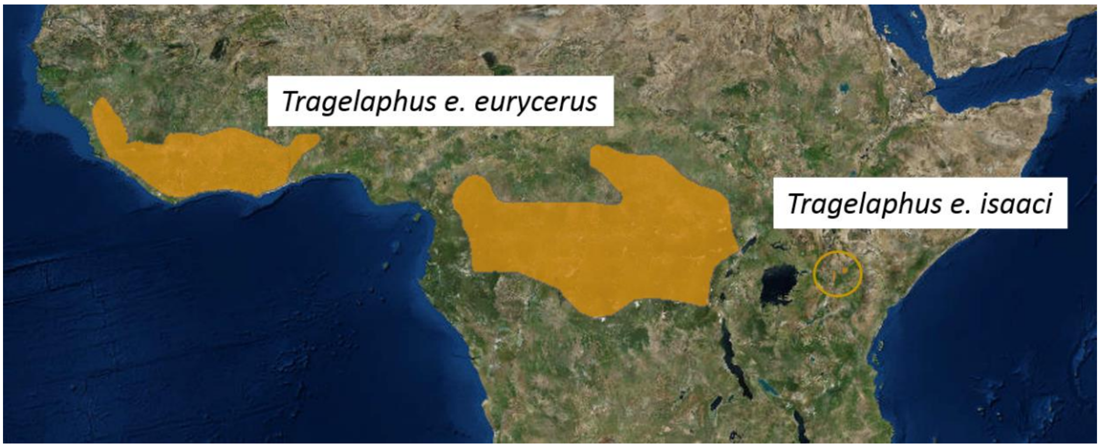

*Figure 2: IUCN Red List map showing the range of Lowland bongo (Tragelaphus e. eurycerus) and mountain bongo (Tragelaphus e. isaaci).*

Bongo distribution was likely already fragmented due to its dependence on these forests, historically occupying the Afromontane ecosystem typical of these highland areas scattered across Kenya and Uganda (Kingdon, 1982). Its range included the Cherangani hills (Price, 1969), the Aberdare Range, Mt. Kenya, and Mt. Elgon (on the border between Kenya and Uganda), and it was typically found throughout the Mau escarpment (Kingdon, 1982), the largest of the Afromontane forests. Further fragmentation due to habitat loss was however inevitable, as the central highlands of Kenya are some of the only areas suited for agriculture in the entire region (Gichuki, 2008; UNEP, 2012). Moreover, the highlands also provided an additional asset for local communities, available pastures for cattle even in periods of drought (Gichuki, 2008). Therefore, the areas occupied by this antelope were prime land for cattle herders, and this caused cattle and bongo to come in in close contact with severe consequences due to the multiple outbreaks of rinderpest, which has impacted heavily on a variety of ungulates, specifically bovines, during the 19th and 20th century (Simonsen et al., 1998; Kock et al., 1999). In addition to habitat loss and disease, bongo were also considered one of the most prized quarry for trophy hunting, due to its large size and difficult terrain in which it is found (Prettejohn, 2012; Gippoliti et al., 2018).

The combination of all these factors have caused a severe decline, and bongo were sighted only in the Aberdares until the 1980's (Kingdon, 1982), with no later sightings recorded. The first evidence of their survival results from the work of a local NGO, the Bongo Surveillance Project (hereafter BSP; Prettejohn, 2008). Since the early 2000s, BSP has conducted surveillance monitoring of areas where bongo were usually found using camera traps placed at salt licks (an example of a camera trap capture is shown in Figure 3). Their work provided evidence in 2004 of the persistence of this antelope in the wild, in the Aberdares, and later found individuals in additional highland forests of central Kenya: Eburu, Mt. Kenya, and Maasai Mau (Figure 4). With less than 100 individuals thought to remain in the wild and its fragmented distribution, the mountain bongo is currently listed as Critically Endangered by IUCN (IUCN SSC Antelope Specialist Group, 2016).

*Figure 3: BSP camera trap capture taken in the Aberdares.*

The scarcity and elusiveness of this antelope made it difficult to study, and valuable information including demographics, habitat, and genetics, were limited, thus hampering the development of effective conservation actions. Nonetheless, information regarding antelope life history traits have long been available through direct observations in the Aberdares (Kingdon, 1982) and in the Lowland bongo (Hillman, 1986; Klaus et al., 1998; Klaus-Hügi et al., 1999, 2000). A series of studies conducted in the early 2000's made a significant contribution to expanding current knowledge on this antelope. These focused on the habitat selection of bongo in the Aberdares and Mt. Kenya (Estes et al., 2008) to inform the development of a distribution model to inform conservation within these two areas (Estes et al., 2010, 2011). Further research provided an initial assessment of the genetic situation, while also confirming the current distribution of the four remnant populations (Faria et al., 2011).

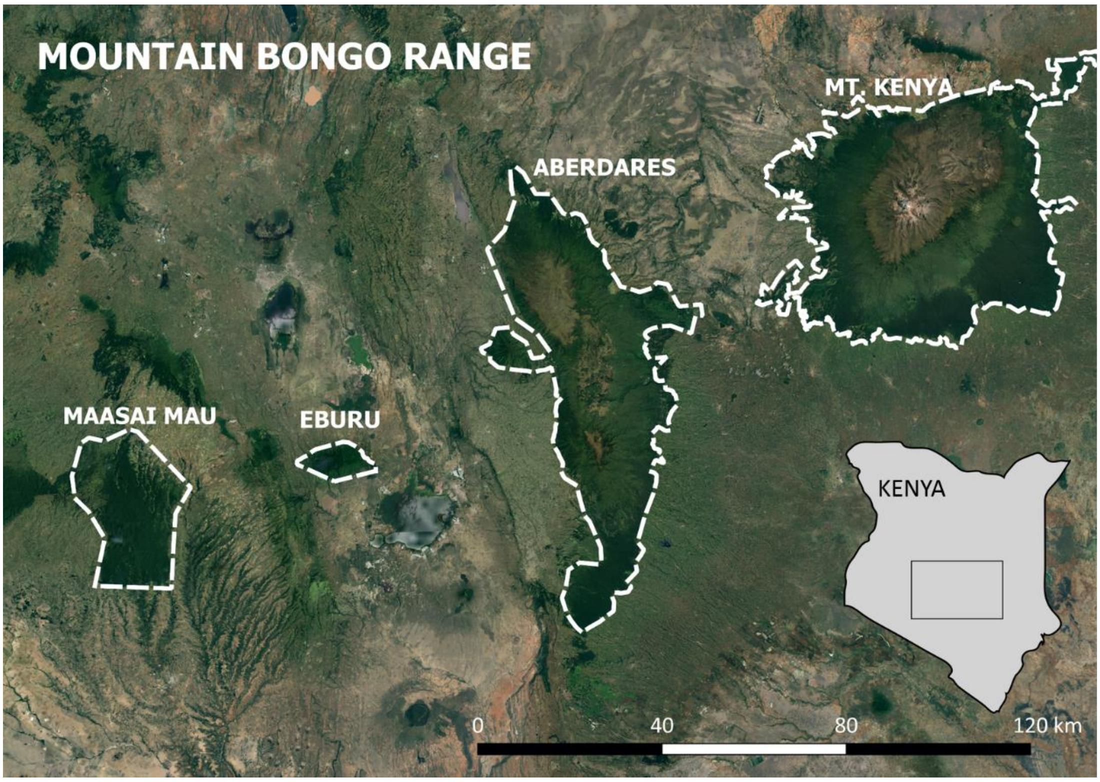

*Figure 4: Map showing the extant range of mountain bongo, consisting of only four mountain areas in central Kenya.*

Estes et al. (2008) provided the first analysis of habitat selection in bongo, although there were limitations to the study. Vegetation, especially presence of herbs and forbs, was found to be the most important predictor for bongo presence. These findings agree with ecological evidence that the bongo is a forest browser specialising on soft and emergent vegetation (Kingdon, 1982). Their work proceeded to develop a species distribution model for bongo (Estes et al., 2011) that provided the first tool available for managers to prioritise areas where bongo may be present, or areas with optimal habitat. However, these studies were conducted with a lack of knowledge on the demographic situation of the populations inhabiting the sampled areas, Aberdares and Mt. Kenya, which were thought to be the most important areas for bongo at the time. The lack of information on the demographics may have caused some areas to be considered optimal when in fact marginal individuals in sub-optimal habitat occupied them, a common issue in developing distribution models for endangered species (Guisan et al., 2017). Therefore, the inclusion of demographics could help refine the predictions by only assessing those areas where populations are thriving (i.e. source populations; Battin, 2004), therefore providing a more reliable tool for managers in deciding where to focus their effort (Osborne and Seddon, 2012).

Faria et al. (2011) measured population genetic variation across wild bongo populations relying on the control region of mitochondrial DNA (mtDNA) relying on faecal samples collected across the highlands of central Kenya. Results confirmed the presence of the antelope in the four areas currently recognised as the extant range: Aberdares, Mt. Kenya, Eburu and Maasai Mau. Moreover, findings showed a very low mtDNA genetic diversity with only two haplotypes identified, compared to the 23 haplotypes found in the sympatric populations of waterbuck (*Kobus ellypsiprimnus*), a similarly sized antelope. Such a low genetic diversity is concerning for the long-term conservation of this antelope, as low genetic variation hampers adaptive potential (Willoughby et al., 2015). Moreover, the low genetic variation found in bongo can indicate that inbreeding is occurring among wild individuals. Inbreeding heightens the probability that individuals will be homozygous for adverse recessive alleles due to common ancestry, this can in turn lead to effects that negatively influence population growth (e.g. higher mortality in juveniles and lower fecundity in adults, Keller and Waller, 2002). The combination of these adverse effects, referred to as inbreeding depression, is of particular relevance for any small population and it has been shown to affect ungulates (Ralls et al., 1979; Ballou and Ralls, 1982). The genetic situation of wild bongo described by Faria et al. (2011), combined with its fragmented distribution and small population size is a cause of concern, as the combination of these aspects heighten its risk of extinction (Frankham, 2005).

Reasons for optimism in the conservation of bongo come from the existence of a captive breeding program. Bongo have been bred in captivity since the 1960's with an international captive breeding program which currently consists of more than 700 individuals in zoos and similar facilities worldwide (Bosley, 2016). Therefore, there is an insurance population in case of an extinction of the remnant wild populations. Nevertheless, captive individuals can have a greater role than a mere insurance to the existence of this antelope, as conservation actions such as reintroductions or reinforcement of existing populations often rely on captive individuals (Armstrong and Seddon, 2008). Therefore, the captive bongo population provides opportunities for the conservation of bongo in the wild, an aspect long recognised in the conservation community (East, 1999). Moreover, the captive individuals might represent a reservoir of genetic variation that could be returned to the wild (Stanton et al., 2015) and the augmented gene flow between captive and wild populations could help wild populations through genetic rescue (Bell et al., 2019).

Genetic variation within the captive bongo population has been studied previously. O’Donoghue (2017) assessed what genetic variation was found in the European zoo population analysing the control region of mtDNA (same as Faria et al., 2011). Samples were collected from 10 individuals hosted in eight institutions. Results from these 10 individuals, claimed to be representative of the founder population, showed only a portion of the genetic variation found in the wild, with only one of the two haplotypes represented. Svengren et al. (2017) used Single Nucleotide Polymorphisms (SNPs) to investigate the genetic variation between the wild population of the Aberdares and a population held in a captive breeding facility in Kenya, the Mount Kenya Wildlife Conservancy (MKWC, Nanyuki Kenya). Their results showed little support for any difference between the two, thus suggesting that limited, if any, unique variation is found in the captive population.

However, while neither study substantially sampled the captive population or the wild population, their results suggest that there is limited, if any, unique genetic variation within the captive population compared to what is found in the wild. Therefore, the opportunities for genetic rescue relying on captive individuals may be limited. Nevertheless, this also means that the risk of outbreeding depression, where the insertion of novel genetic variants in a population causes similar effects to those caused by inbreeding, may be limited for bongo. Nevertheless, considering the critical situation of bongo in the wild, a deeper comparison between captive and wild populations, with wider coverage of both, is needed to identify the best individuals and lineages for reintroduction. Plans for reintroduction started in the early 2000's with the bongo repatriation project (Reillo, 2002; Veasey, 2010), where 18 individuals from North American zoos and other institutions were translocated to MKWC in 2004. The aim of the project was to genetically reinforce the resident breeding stock and produce offspring that would then be released in the wild. While the reintroduction of captive individuals did not proceed, the repatriation project managed to reinforce the captive population at MKWC, which currently counts ~ 70 individuals. This population, due to its size and proximity to bongo native range, may provide suitable individuals for reintroduction projects.

The information gathered through the studies reported failed however to translate into additional conservation actions. Therefore, there is a need for updated information on the current situation of wild bongo, firstly through a better understanding of their demography. Understanding the situation of the wild population would then allow a refined analysis of habitat selection and predict distribution of optimal habitat. Besides, bongo conservation would benefit from an augmented knowledge on bongo genetics in both wild and captivity. Conservation has made already a difference for many ungulate species (Hoffmann et al., 2015), it is therefore imperative that conservation actions are designed, and implemented, for the conservation of this iconic antelope, which could, and should, become a flagship for the entire Afromontane ecosystem of eastern Africa.

## Thesis Aim

The aim of the project presented here is to provide information to managers and conservationist working for the preservation of this iconic antelope, through the study of bongo ecology and genetics. In order to do so, I first focus on assessing the demographics of the remnant population. Understanding the structure and dynamics of the populations would then inform the development of an updated Species Distribution Model that focuses on breeding and growing populations for its predictions. Furthermore, I will compare the genetic diversity found in the remnant populations with that of captive individuals from European zoos and MKWC to also address the concerns regarding outbreeding depression. An investigation into the relevance that the founders may have had on the current genetic makeup of the captive population concludes the project. The information would refine and augment current knowledge on the situation of bongo in the wild, while also contributing to assess the relevance and role the captive population could play in its long-term conservation.

## Thesis Description

I report the project in five data chapters:

In Chapter 2**,** I describe a novel visual ID system for bongo to implement in camera trap-footage. The system is tested for being informative (i.e. effective in discerning individuals) and reliable among multiple observers. I also present the development of a quantitative tool to help recognise bongo spoor in the field, a useful tool for trackers and researchers alike.

In Chapter 3, I assess the demographic status of the remnant wild bongo populations implementing the ID-system presented in Chapter 2 on extensive BSP footage covering all four extant populations in the period 2013 – 2018. Using Mark-Recapture methods, I report the first estimates for both census size, and sex and age specific vital rates in the remnant populations.

In Chapter 4, I report a species distribution model to identify areas of bongo habitat. Here, sampling was conducted only in Aberdares and Maasai Mau following results from Chapter 3, which indicate that these are the only areas where breeding herds are present. I utilise presence and absence locations from multiple surveys conducted in these areas using both camera trapping and sign surveys. I rely on these locations to build a Random Forest model to predict habitat suitability throughout the current bongo range.

In Chapter 5, I measure and report the genetic makeup of two wild bongo populations (Aberdares and Maasai Mau) for the mtDNA control region. I compare this with samples from MKWC, in order to augment previous genetic information about this captive population, which is likely the most relevant for conservation actions such as reintroduction, with the aim to elucidate whether a risk of outbreeding depression exists. I include in the analysis European individuals sampled in previous studies to provide a wider coverage of the current genetic diversity found in captivity.

In Chapter 6, simulations of both pedigrees and genotypes are used to try to decipher the reason for the low diversity encountered in the bongo captive population. I therefore investigate the influence of founder relatedness with respect to inbreeding in a large, closed population. The bongo is an optimal case study as its pedigree is well documented since the beginning of the breeding program, while recent measures of relatedness of captive individuals are also available thanks to recent studies.

The key findings gathered through this project are presented in a concluding chapter where I discuss them in the context of practical conservation management for the bongo.

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# **Chapter 2**:
# A Visual ID System and Track Identification Tool to Facilitate Monitoring of the Critically Endangered Mountain Bongo

### ***Context in the Thesis***

*The chapter focuses on developing a visual ID system to implement on camera trap footage. The system is tested for its information content and for its reliability among multiple observers. The system is then implemented in Chapter 3 to conduct Mark-Recapture analysis. Moreover, the chapter explains the analysis of bongo and waterbuck spoor to try and identify a measure by which the tracks of these antelopes can easily be distinguished.*

*A slightly modified version of this chapter is included as an appendix to the Mountain Bongo National Strategy and Action Plan (KWS, 2019) (Appendix 1).*

### **Abstract**

Conservation of the critically endangered mountain bongo (*Tragelaphus eurycerus isaaci*), an antelope endemic to Kenya, depends on reliable information on the ecology of remnant wild populations. Monitoring these populations would provide such information, but it has been hampered by the lack of reliable tools for individual identification and spoor assignment. Here, we rely on a captive population to develop a visual identification system for camera trap footage, and a quantitative tool to identify bongo spoor in the field. We implement an information theoretic approach to assess the most informative visual features of 67 bongo captive individuals hosted at Mount Kenya Wildlife Conservancy in Kenya, which we used to develop an identification system. We further test the system for its reliability among observers using Kappa statistics. We also compared the measurements of 100 bongo footprints with 50 of waterbuck (*Kobus ellypsiprimnus*) to discern between the two antelopes in the field. We obtained an identification system that relies on three informative features (stripe pattern, facial markings and horns appearance) with a substantial reliability among different observers (Light’s Kappa = 0.64). We also show that the aspect ratio of footprints can help assign species between bongo and waterbuck, with a measure of 1.22 (± 0.08) for bongo and 1.49 (± 0.10) for waterbuck. The tools developed here can help managers and field workers in the study of this rare antelope. Moreover, such reliable tools allow monitoring activities to be less dependent on individual expertise, and thus permit consistent monitoring of bongo remnant populations.

## Introduction

The global loss of biodiversity requires effective conservation in order to avoid species extinction (Butchart, Walpole and Coll, 2011). Captive populations play a prominent role in the long-term conservation of threatened species (Conde *et al.*, 2011) and integrating captive and wild populations is the ultimate aim of the One Plan approach (Lees and Schwitzer, 2013). One of the benefits of such approach is the importance of captive populations to conduct research to inform and drive conservation actions in the wild, as species conservation depends on knowledge of wild populations (Armstrong and Seddon, 2008). Information relevant for conservation includes knowledge of population structure and dynamics, usually ascertained through monitoring programs (Nichols and Williams, 2006), and a thorough understanding of its habitat and distribution (Guisan et al., 2013).

Information retrieved through monitoring is valuable for designing effective conservation actions (Nichols and Williams, 2006). A powerful method for population monitoring is mark-recapture (MR), as it allows estimation of vital population parameters such as survivorship, recruitment and population growth rate (Lebreton, Pradel and Clobert, 1993; Pradel, 1996). MR requires individuals either to be physically marked or to be identifiable noninvasively by using, for example, unique natural markings or scars (Petit and Valiere, 2006). Camera trapping (CT) has become a popular tool in conservation monitoring, allowing the continuous sampling of an area of interest with limited effort, while also enhancing the chances of encountering rare and elusive species (Nichols and Ullas Karanth, 2011; Rovero et al. 2014). Examples of MR analysis through CT footage are predominantly limited to large felids (Karanth and Nichols, 1998; Karanth *et al.*, 2006; Soisalo & Cavalcanti, 2006; Alexander *et al.*, 2016; Weingarth *et al*., 2012; Harmsen *et al*., 2017). In these studies, either a single observer identifies individuals or multiple observers analyse the footage where encountered animals are assigned an identity depending on their agreement (Rich *et al*., 2014; Alexander *et al.*, 2016). This approach relies on observer expertise in individual identification from photographs, which may be non-repeatable or time-consuming (e.g., see Hiby *et al*., 2009). Moreover, reliance on observer expertise not only hampers the immediate replicability of the analysis, but it also affects the likelihood that a monitoring program may continue in time if expertise changes or is lost, a major recurrent issue in many conservation monitoring programs (Legg and Nagy, 2006).

Software automation can alleviate some of the requirements for human expertise in individual identification using photographs, and promises to improve the effectiveness and speed of individual identification (Hiby *et al.*, 2009; Bolger *et al*., 2012). Software automation has been successfully implemented in MR studies (Morrison *et al.,* 2011; Bolger *et al*., 2012; Zero *et al.*, 2013; Jiang *et al*. 2015) and may allow for the matching of individual patterns (e.g., spots, stripes or scars) by evaluating similarity between two or more images. Some of the available software is optimised for single species (e.g. ExtractCompare, www.conservationresearch.org.uk, is currently optimised for 20 species, from whale shark to crested newt among others), while others can be used with any species that presents individual specific patterns (e.g., WILD.ID, Bolger *et al*., 2012; HotSpotter, Crall *et al*., 2013). The majority of automated systems rely on *ad hoc* photographs. This hinders their application to ongoing monitoring programs that were not designed for individual identification. Thus, while automated software for processing photographs is promising, at present it still presents considerable challenges for individual identification in conservation monitoring. Nevertheless, methods exist that allow identifying individuals using natural markings from footage, while not relying extensively on the observer expertise or software, by using features that vary among individuals by shape, size or other attributes (Pennycuick, 1978). This approach allows individual identification to be repeatable and objective, while still easy to implement on camera trap footage. However, for such a system to be useful for monitoring it should be efficient in discerning individuals (i.e. informative) and it should be repeatable amongst different observers (i.e. reliable). Reliance on known individuals in a captive setting would allow for the development and rigorous assessment of such a system.

Captive individuals can also provide a valuable source of information vital to field workers when it comes to identifying spoor, as locating sites of occurrence of wild populations often depends on finding evidence of their presence, such as tracks and dung. However, this is usually dependent on fieldworker expertise or that of their team (Stander et al., 2009). The use of spoor for these studies is particularly common when studying species that are difficult to encounter and live in areas where direct sightings are rare. Difficulties in distinguishing dung among different species are common without the implementation of genetic barcoding, particularly in forest habitat (Bowkett et al., 2009). Reliance on tracks can be an alternative, but the lack of any assessment for species identity make it less rigorous. Access to captive individuals would allow for the testing of measures and characteristics of a species spoor prior to fieldwork, thus providing practitioners with information that would minimise the reliance on local expertise, which although valuable, could be limited when studying rare and elusive species.

The mountain bongo (*Tragelaphus eurycerus isaaci*, hereafter bongo) is a large forest antelope endemic to the Afromontane forests of central Kenya (East, 1999). Currently limited to four areas (Faria *et al*., 2011, Elkan & Smith, 2013) and with an estimate of less than 100 left in the wild, IUCN considers this subspecies Critically Endangered (IUCN SSC Antelope Specialist Group, 2016). Moreover, since the early 2000's the Bongo Surveillance

Program (www.mountainbongo.org, hereafter BSP, Prettejohn, 2008) has been conducting surveillance monitoring using camera traps set in strategic locations across the known wild bongo range. However, bongo monitoring is limited to surveillance of these remnant populations, where the persistence of populations is assessed without further insights into their structure and size. This is due to the lack of an efficient Identification system, which does not allow deeper analysis. An identification system would enhance the information retrievable from BSP footage. Therefore, the use of a reliable identification system could positively affect knowledge and conservation management of wild bongo. Individual bongo possess multiple markings on flanks, chest and limbs (Elkan & Smith, 2013), and there is evidence these markings are informative for individual identification (Gibbon *et al.*, 2015). Hence, an identification system for this antelope can be developed relying on visual features. The use of a system that relies on objective features would further alleviate the individual expertise needed for identification and, if repeatable, could readily be implemented by different observers on any available footage. The basic requirements for such a system are ease of use and reliability amongst observers with little or no expertise.

An additional problem when studying bongo in its native range is the co-occurrence of the similarly sized waterbuck (*Kobus ellipsiprymnus*). As bongo direct sightings are virtually non-existent, fieldwork relies on spoor (namely tracks and dung) to assess its presence in a location. Even experienced trackers can easily confound waterbuck and bongo dung with up to 30 % misidentification rate (Faria *et al.,* 2011). Whereas, bongo and waterbuck tracks are considered easier to distinguish due to differences in shape, nevertheless, field workers need to rely on experienced trackers (Estes *et al*., 2008), due to the lack of quantitative measures to facilitate species identification. While tracks can be reliably used to assess species presence (Stander et al., 2009) the difficulty in encountering the bongo in the wild leads to a very limited amount of information regarding its spoor, hence even the expertise of trackers may not be extensive.

While wild populations are in a critical situation, the *ex-situ* conservation of bongo has been successful, with a captive breeding program initiated in the late 60's and that now comprises a world population of over 700 animals (Bosley, 2016). Captive individuals can prove beneficial to their wild counterpart in allowing access to otherwise difficult to study species (Mendelson III, Schuett and Lawson, 2019). Therefore, issues encountered while studying populations in the wild can be overcome by relying on captive individuals to develop methods and tools to then implement in field studies (Hutchins and Conway, 1995). Access to captive individuals can therefore greatly help field studies on bongo, and thus help conservation actions. Bongo monitoring efforts would benefit from the development of a quantitative tool to discern spoor from waterbuck. Hence, both the lack of an ID system to conduct monitoring from camera trap footage, and a reliable tool to easily identify spoor represents a challenge for *in-situ* conservation of this iconic antelope.

Here I rely on a large captive populations of bongo (~60 individuals at the time of this research) at Kenya at the Mount Kenya Wildlife Conservancy (MKWC, www.animalorphanagekenya.org) to develop an informative individual identification system that is repeatable and requires little training, and a measure to help in differentiating tracks between bongo and waterbuck. My objectives were 1) to identify visual features that contain the greatest variation between individuals in bongo; 2) to devise an identification system for these features; 3) to test the repeatability of our identification system among multiple naïve observers; and 4) to develop a quantitative way to distinguish bongo and waterbuck spoor. I discuss my results in the context of relying on captive populations to design effective tools to help field workers in monitoring rare and elusive species.

## Methods

### *ID-System Development*

The bongo is characterised by 9-15 distinctive lateral stripes and variable white markings on cheeks, chest and limbs (Elkan & Smith 2013). These features can be used to distinguish individual bongo (Gibbon et al. 2015). However, these markings have not been used in individual bongo identification. In order to design an informative and reliable identification system I relied on the captive individuals held at MKWC. Photographs of the 61 individuals of both sexes aged between 2 months and 16 years were assessed in August 2016.  As the coat pattern in bongo is bilaterally asymmetrical, a unique identification must be made for each flank (i.e., two identified flanks per individual). Therefore, each individual flank (N = 122) was photographed and a library of MKWC bongo flanks was created.

An identification system based on natural markings relies on a set of features (i.e. visual markings) that are independent of one another and show enough variation in their appearance to be assigned at least two variants (Pennycuick 1978). The visual markings I considered to use as features for the identification system were the facial markings, the stripes on the flank, and the state of the horns (e.g. normal, broken or bent). These markings are visible in camera trap footage with both daylight and infrared-light at night. Besides, the candidate markings are independent of one another. Different characteristics of each of the three features were considered for developing the ID-system: number and shape of facial markings; number of stripes; the pattern of individual stripes; and the state of the horns. . We adapted the work of Petersen (1972) in defining a code system for the identification of zebras (*Equus quagga*) as a template for our system, so to translate individual bongo patterns into individual identification codes. The objective was to find a system that would assign a unique code to each flank. I followed an iterative approach in testing the various characteristics of the candidate features in order to simplify the code.

Multiple rounds of coding were implemented where a single characteristic of a feature was arbitrarily excluded and flanks re-coded accordingly. If the new code could not uniquely identify the flanks, it was discarded and a different version was tested.

### *ID-System Information Content*

Information theory allows assessing the effectiveness of a system in identifying individuals by providing a measure of the risk of finding a duplicate with the same characteristics in a population of a certain size (Pennycuick & Rudnai 1970). The risk of finding a duplicate is related to the information contained in an individual code, which is measured in bits (Pennycuick 1978). Implementing an information theoretic approach, I could thus assess the efficacy of each feature chosen for the system in unequivocally identifying individuals also in the wild. While no census of bongo populations has ever been conducted, it is estimated that none of the remnant populations exceeds 50 individuals (IUCN SSC Antelope Specialist Group, 2016). Therefore, I considered a system reliable for use in the wild if, on average, the individual identifications retained enough information to avoid finding duplicates in a population of 50. My approach relies on the assumption that the variety in this large captive population is comparable to that found in the smaller wild populations. Following Pennycuick & Rudnai (1970), I evaluated the information content of each code given to individuals by summing the bits contained in each variant of a feature (e.g. broken horns) in the identification system using equation (1).

$$\log_2(1/Fi) = Ii \qquad (1)$$

where $Ii$ is the information content (i.e. bits) of variant $i$ of a feature, and $Fi$ is the proportion of occurrence of variant $i$ amongst the assessed individuals. Hence, the less frequent a variant of a feature the more informative it becomes in identifying individuals. The higher the information content, the lower the risk of finding an individual with the same code. In order to avoid a duplicate in a population of 50 with odds of 1:1000 we need an information content of 10 bits (Pennycuick 1978).

### *ID-System Reliability*

To test the identification system repeatability amongst different users I assessed Inter-rater reliability (IRR), defined as a way of quantifying the degree of agreement between two or more coders who make independent ratings about the characteristics of a set of subjects (Hallgren 2012). In our case, the subjects will be bongo individual flanks and the characteristics will be the features of the identification system. IRR is evaluated using an index of concordance, usually Cohen's kappa (Cohen, 1960). Here I implemented Light's kappa (hereafter kappa), a variation of Cohen's kappa allowing the evaluation of concordance among multiple observers (Light 1971; Hallgren 2012). Landis and Koch (1977) provide an arbitrary scale to evaluate IRR: kappa = 0.0 to 0.2, slight agreement; 0.21 to 0.40, fair agreement; 0.41 to 0.60, moderate agreement; 0.61 to 0.80, substantial agreement; 0.81 to 1.0, almost perfect to perfect agreement.

I presented a sample of 10 pictures of bongo flanks (from camera traps and captivity) to 15 naïve observers along with brief instructions and four example bongo flank photographs with assigned codes (codes for these were assigned by Tommy Sandri). Observers were to assign a code to each of the 10 test flanks. Observers had no prior experience in using the identification system nor were they experienced with bongo. I evaluated IRR among the 15 observers for each feature of the system (e.g. concordance amongst observers in coding facial markings, stripe pattern and horn state) on each of the 10 individual bongo flanks. The resulting kappa is the overall agreement of the 15 observers on coding a flank according to the system features. This results in 10 different values of kappa (one for each bongo flank in the test) which I interpret as the reliability of the system in coding an individual among different observers. In order to obtain an average value of kappa for my

ID-system, I calculated the arithmetic mean of the 10 resulting values for kappa, which I interpret as the overall reliability of the system in coding different individuals amongst different observers. All analyses were conducted in R (R Core Team, 2019) using the R package {irr} (Gamer et al., 2019).

### *Spoor Identification*

Tracks were searched within pens where bongo are the only ungulate present in the MKWC. Maximum length and width were measured using a caliper accurate to 0.01 cm by a single observer, as measures can vary significantly among observers even when using precision calipers (Bowkett et al., 2013). Subsequently, in order to account for differences in shape, the aspect ratio (max length/ max width) was calculated. Waterbuck spoor was collected in areas inaccessible to the captive bongo herds. Waterbuck in MKWC are free roaming, therefore, the antelopes were tracked with the help of a local guide in search for tracks. These were only measured following direct sightings of waterbuck, so that the origin of tracks was certain. Tracks were located in multiple terrain types, from forest areas to sand, open grassland, and mud, thus accounting for differences in terrain texture. In total, 150 tracks were measured: 100 bongo and 50 waterbuck. In order to assess any significant difference in the measurements and find a good predictor for species identification I implemented a stepwise approach: errors of every variable (length, width, aspect ratio) were screened for normality, while homoscedasticity of data between the two groups (bongo and waterbuck) was also tested. Variables with equal variance were then tested for significant differences using a two-sample t-test. The measurements found to differ significantly between the two species were then implemented in a logistic regression to test the efficacy of the measure in discerning bongo and waterbuck. The predictive ability of the model built using a training dataset containing 70% of all observations, was assessed on a test dataset using the area under the curve (AUC) of a Receiver – Operator

Characteristics (ROC) (Fielding and Bell, 1997). Such measure spans from 0.5 (predictive ability equal to random assignment) to 1 (maximum accuracy in prediction). To further assess model reliability compared to random chance I implemented Cohen's kappa (Cohen, 1960). Moreover, the 95% confidence intervals of the mean of measurements in each species were calculated. In case of no overlap between the confidence intervals, the measure was deemed safe to implement in the field. All analyses were conducted in R (R Core Team, 2019).

## Results

### *ID-System Development*

I needed five rounds of coding, elimination of a characteristic of a visual feature and re-coding, to find an identification system effective in distinguishing MKWC individuals using the three chosen features (horns, stripes and facial markings) while reliant on the lowest number of characteristics of each feature. The characteristics fundamental to code distinctly the flanks of the individuals from MKWC were: number of facial markings (*2* or *3*), shape of the uppermost cheek marking (*r*, round or *nr*, not round), state of the horns (*hn*, normal, *hcr*, crossed, *hbr*, broken, or *hb*, bent) and the stripe pattern. A combination of letters and numbers translates the latter in a code. Letters indicate peculiar patterns on the flank: for example *v* (two stripes converge), *y* (one stripe bifurcates); roman numerals indicate stripes that appear narrower than the others on the flank do (e.g., *II* indicates two narrow stripes). Arabic numerals identify the number of stripes interposed between peculiar patterns, if present, or simply identify the number of stripes on a flank in case of no peculiar pattern. Individual codes include the portrayed flank side, *L* for left and *R* for right and the sex of the portrayed individual, *F* for female and *M* for male. We can assign a sex to individual flanks, as like other *Tragelaphus* species mature males are larger and darker in colour whereas horns, although present in both sexes, appear narrower in females (Elkan & Smith 2013). I excluded sex from the calculation on information content of the system, as the frequency of males and females held at MKWC likely differs to that found in the wild. Figure 1 illustrates the main features of the identification system with two exemplary bongo flanks.

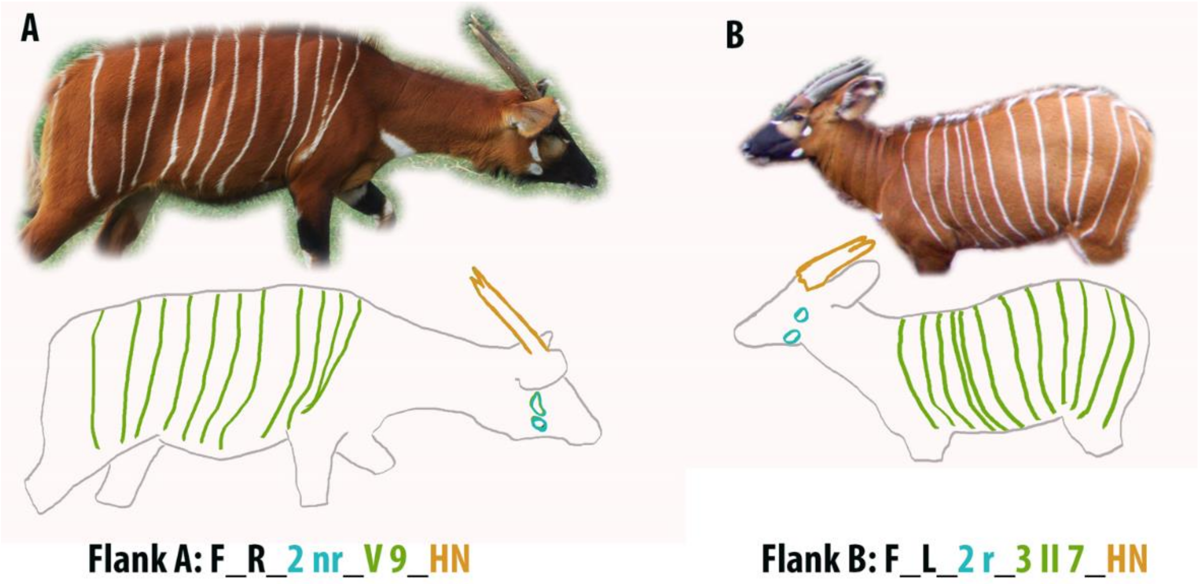


*Figure 1: Example of our newly developed ID-system for bongo flanks: Flank A is coded as F (female), R (right flank), 2 nr (two facial spots, upper spot is not round), V (two stripes converge), 9 (nine stripes with no peculiar feature), HN (horns appear normal). Flank B is coded as F (female), L (left flank) 2r (two facial spots, upper is round), 3 (three stripes with no peculiar feature), II (two stripes appear narrower than the others on the portrayed flank), 7 (seven stripes with no peculiar feature), HN (horns appear normal).*

### *ID-System Information Content*

The features vary in their information content, with the stripe pattern being the most informative. The average information content of each of the three features included in the ID system is shown in Table 1. The average amount of information retained in each individual code is 10.24 (± SE 2.5) with a minimum value of 6.07 and a maximum of 18.57 (Figure 2). More than half of the flanks codes (N= 63) fall above the 10 bits threshold, which, according to Pennycuick (1978) is the information needed to avoid duplication in a population of 50 with a 1:1000 risk. Besides, 80% of all codes (N= 99) have an information content above 8 bits, which means a risk of 1:100 of duplication in a population of 50 (Pennycuick 1978). Therefore, I consider our system robust enough for field applications, where no population is expected to include more than 50 individuals (IUCN SSC Antelope Specialist Group, 2016).

*Table 1: Values of Light's Kappa among 15 naïve observers, and average information content (expressed in bits) of each feature of the ID system.*

| Feature | Light's Kappa | Information |
|---|---|---|
| Horn State | 0.52 | 4.24 (±1.66) |
| Facial Markings | 0.72 | 4.12 (±2.16) |
| Stripe Pattern | 0.44 | 4.96 (±1.23) |

### *ID-System Reliability*

IRR analysis on 15 naïve observers on 10 pictures of bongos show a substantial agreement (mean kappa = 0.66 ± 0.14). The lowest individual score of agreement is 0.45, a moderate agreement, with the highest being 0.84, almost perfect agreement. More specifically, five of the 10 bongo pictures show a moderate agreement amongst observers (0.4 < kappa < 0.6), three a substantial agreement (0.6 < kappa < 0.8) and two an almost perfect agreement (kappa > 0.8) (Figure 3). Thus, all the individual codes show a level of agreement higher than what would be expected by chance (kappa = 0). Thanks to the approach implemented in assessing IRR I am able to identify the most reliable feature among the three used in the identification system. Facial markings and horn state appear to have a high agreement while stripe pattern is the feature coded most differently amongst observers (Table 1). Our results show that the developed ID-system is consistent among different naïve observers, and fits our need for a reliable system readily implementable for use in the field with minimal training.

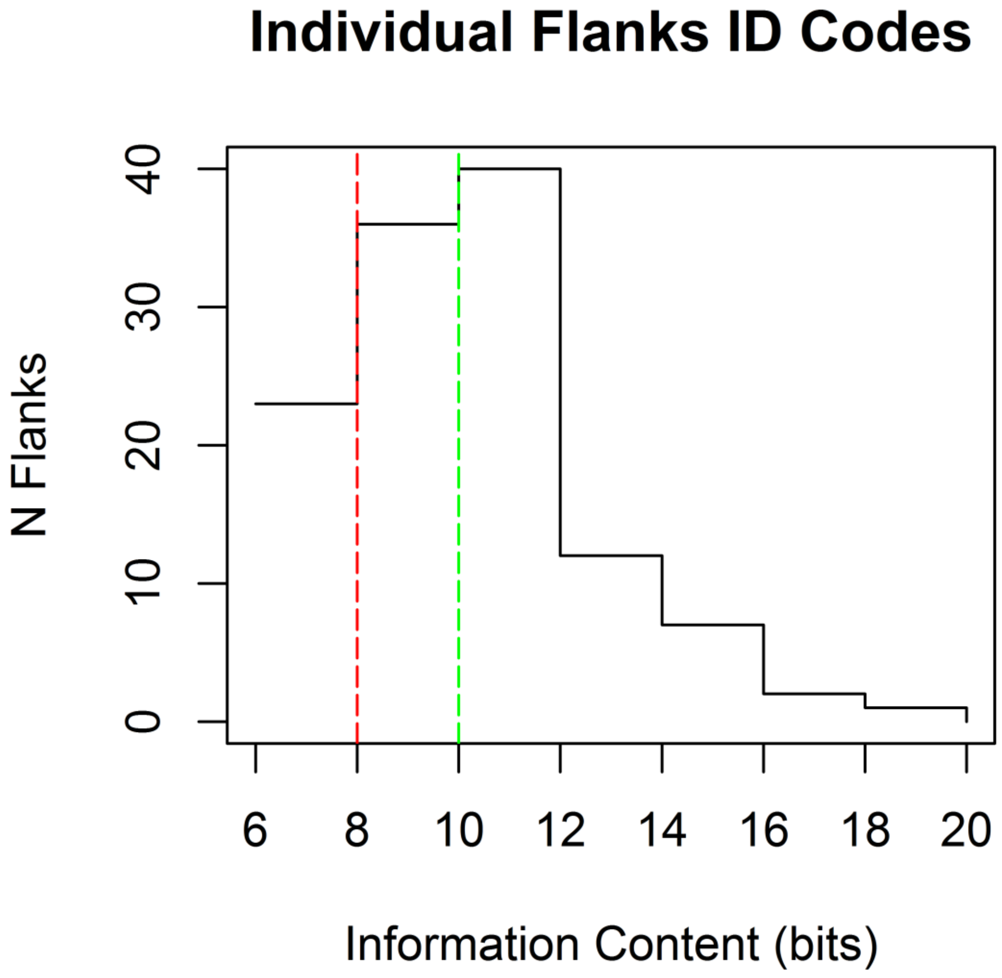
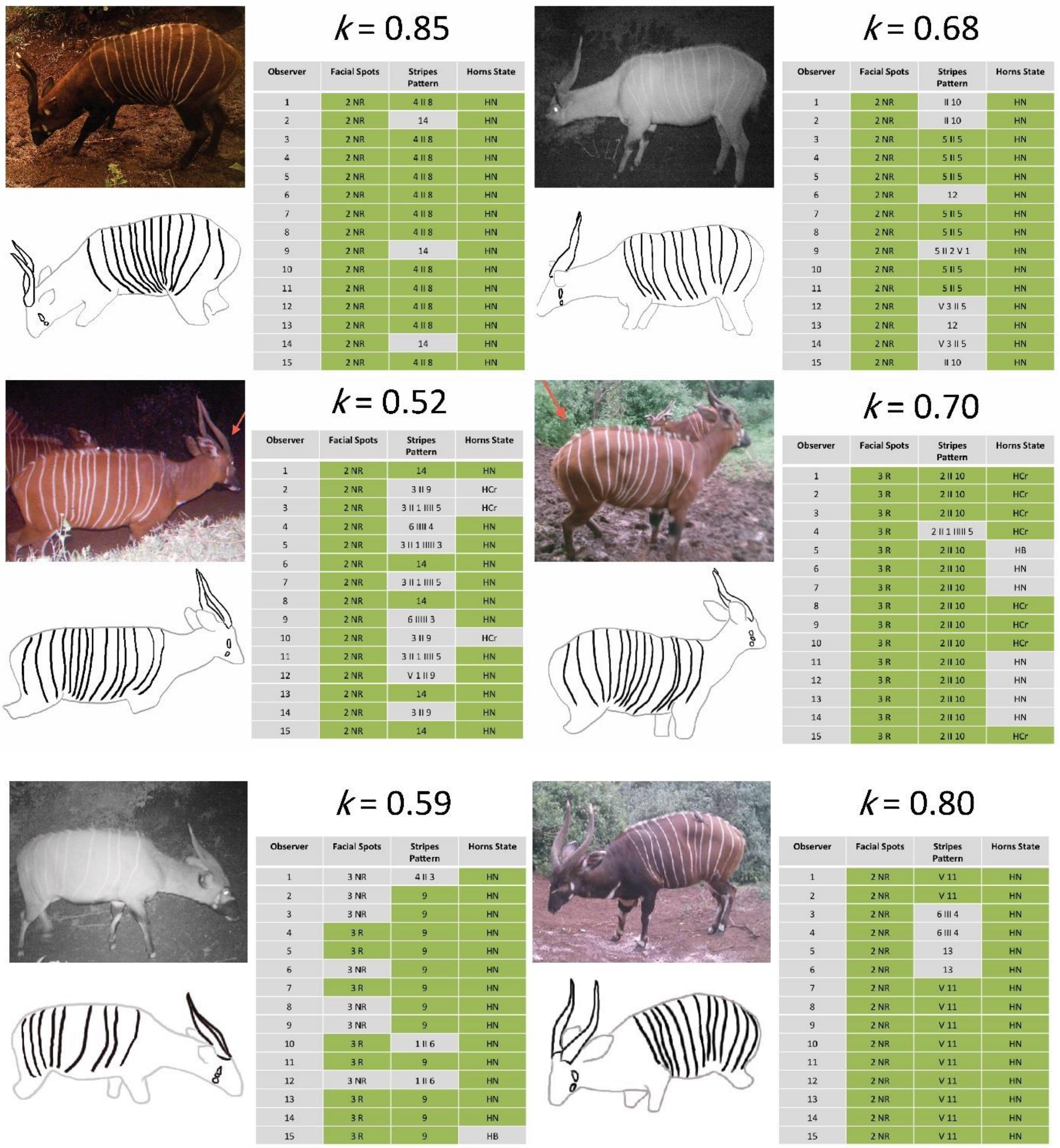


*Figure 2: The graph shows the information (expressed in bits) retained in individual ID-codes of the MKWC bongo (left and right flanks). The dotted red and green lines indicate the threshold for encountering duplicates in a population of 50 with a risk of 1:100 and 1:1000 respectively.*

### *Spoor Identification*

Of the three measures retrieved from tracks (length, width, and aspect ratio), only the latter is homoscedastic between bongo and waterbuck, and resulted significantly different between the two species (Two Sample t-test $p < 0.05$). The different variance in both length and width between the two species was expected, as bongo tracks were sampled covering multiple age classes and sexes, whereas most of the 50 waterbuck tracks were of adult individuals.

I constructed a single model including aspect ratio as a predictor of species. This model shows a good predictive ability (AUC > 0.80) and, with a kappa of 0.77, it can be considered reliable (Landis & Koch, 1977). Although measured aspect ratios overlap in part of my sample (Figure 4) the confidence intervals of the mean do not overlap (bongo= 1.22 ± 0.08; waterbuck= 1.49 ± 0.10).



$k = 0.85$

| Observer | Facial Spots | Stripes Pattern | Horns State |
|---|---|---|---|
| 1 | 2 NR | 4 II 8 | HN |
| 2 | 2 NR | 14 | HN |
| 3 | 2 NR | 4 II 8 | HN |
| 4 | 2 NR | 4 II 8 | HN |
| 5 | 2 NR | 4 II 8 | HN |
| 6 | 2 NR | 4 II 8 | HN |
| 7 | 2 NR | 4 II 8 | HN |
| 8 | 2 NR | 4 II 8 | HN |
| 9 | 2 NR | 14 | HN |
| 10 | 2 NR | 4 II 8 | HN |
| 11 | 2 NR | 4 II 8 | HN |
| 12 | 2 NR | 4 II 8 | HN |
| 13 | 2 NR | 4 II 8 | HN |
| 14 | 2 NR | 14 | HN |
| 15 | 2 NR | 4 II 8 | HN |

$k = 0.68$

| Observer | Facial Spots | Stripes Pattern | Horns State |
|---|---|---|---|
| 1 | 2 NR | II 10 | HN |
| 2 | 2 NR | II 10 | HN |
| 3 | 2 NR | 5 II 5 | HN |
| 4 | 2 NR | 5 II 5 | HN |
| 5 | 2 NR | 5 II 5 | HN |
| 6 | 2 NR | 12 | HN |
| 7 | 2 NR | 5 II 5 | HN |
| 8 | 2 NR | 5 II 5 | HN |
| 9 | 2 NR | 5 II 2 V 1 | HN |
| 10 | 2 NR | 5 II 5 | HN |
| 11 | 2 NR | 5 II 5 | HN |
| 12 | 2 NR | V 3 II 5 | HN |
| 13 | 2 NR | 12 | HN |
| 14 | 2 NR | V 3 II 5 | HN |
| 15 | 2 NR | II 10 | HN |

$k = 0.52$

| Observer | Facial Spots | Stripes Pattern | Horns State |
|---|---|---|---|
| 1 | 2 NR | 14 | HN |
| 2 | 2 NR | 3 II 9 | HCr |
| 3 | 2 NR | 3 II 1 IIII 5 | HCr |
| 4 | 2 NR | 6 IIII 4 | HN |
| 5 | 2 NR | 3 II 1 IIIII 3 | HN |
| 6 | 2 NR | 14 | HN |
| 7 | 2 NR | 3 II 1 IIII 5 | HN |
| 8 | 2 NR | 14 | HN |
| 9 | 2 NR | 6 IIIII 3 | HN |
| 10 | 2 NR | 3 II 9 | HCr |
| 11 | 2 NR | 3 II 1 IIII 5 | HN |
| 12 | 2 NR | V 1 II 9 | HN |
| 13 | 2 NR | 14 | HN |
| 14 | 2 NR | 3 II 9 | HN |
| 15 | 2 NR | 14 | HN |

$k = 0.70$

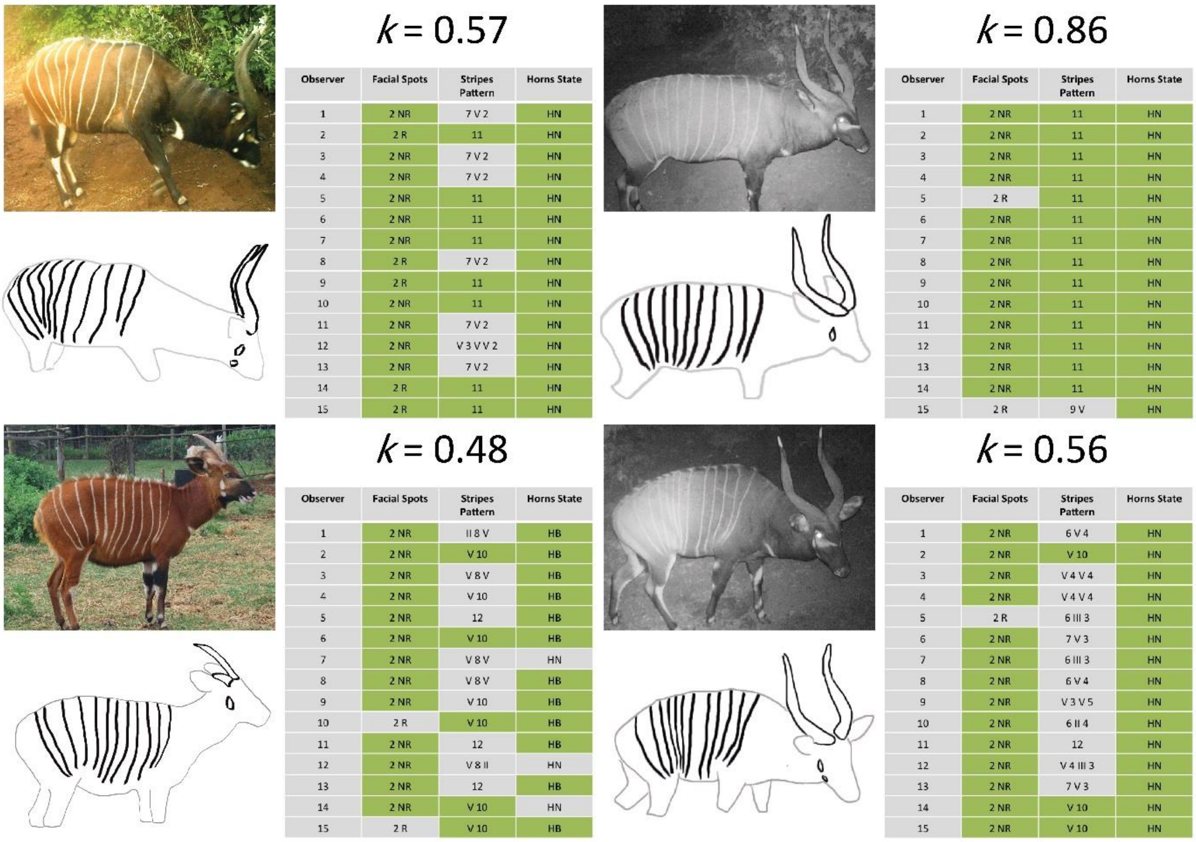
| Observer | Facial Spots | Stripes Pattern | Horns State |
|---|---|---|---|
| 1 | 3 R | 2 II 10 | HCr |
| 2 | 3 R | 2 II 10 | HCr |
| 3 | 3 R | 2 II 10 | HCr |
| 4 | 3 R | 2 II 1 IIIII 5 | HCr |
| 5 | 3 R | 2 II 10 | HB |
| 6 | 3 R | 2 II 10 | HN |
| 7 | 3 R | 2 II 10 | HN |
| 8 | 3 R | 2 II 10 | HCr |
| 9 | 3 R | 2 II 10 | HCr |
| 10 | 3 R | 2 II 10 | HCr |
| 11 | 3 R | 2 II 10 | HN |
| 12 | 3 R | 2 II 10 | HN |
| 13 | 3 R | 2 II 10 | HN |
| 14 | 3 R | 2 II 10 | HN |
| 15 | 3 R | 2 II 10 | HCr |

$k = 0.59$

| Observer | Facial Spots | Stripes Pattern | Horns State |
|---|---|---|---|
| 1 | 3 NR | 4 II 3 | HN |
| 2 | 3 NR | 9 | HN |
| 3 | 3 NR | 9 | HN |
| 4 | 3 R | 9 | HN |
| 5 | 3 R | 9 | HN |
| 6 | 3 NR | 9 | HN |
| 7 | 3 R | 9 | HN |
| 8 | 3 NR | 9 | HN |
| 9 | 3 NR | 9 | HN |
| 10 | 3 R | 1 II 6 | HN |
| 11 | 3 R | 9 | HN |
| 12 | 3 NR | 1 II 6 | HN |
| 13 | 3 R | 9 | HN |
| 14 | 3 R | 9 | HN |
| 15 | 3 R | 9 | HB |

$k = 0.80$

| Observer | Facial Spots | Stripes Pattern | Horns State |
|---|---|---|---|
| 1 | 2 NR | V 11 | HN |
| 2 | 2 NR | V 11 | HN |
| 3 | 2 NR | 6 III 4 | HN |
| 4 | 2 NR | 6 III 4 | HN |
| 5 | 2 NR | 13 | HN |
| 6 | 2 NR | 13 | HN |
| 7 | 2 NR | V 11 | HN |
| 8 | 2 NR | V 11 | HN |
| 9 | 2 NR | V 11 | HN |
| 10 | 2 NR | V 11 | HN |
| 11 | 2 NR | V 11 | HN |
| 12 | 2 NR | V 11 | HN |
| 13 | 2 NR | V 11 | HN |
| 14 | 2 NR | V 11 | HN |
| 15 | 2 NR | V 11 | HN |



$k = 0.57$

| Observer | Facial Spots | Stripes Pattern | Horns State |
|---|---|---|---|
| 1 | 2 NR | 7 V 2 | HN |
| 2 | 2 R | 11 | HN |
| 3 | 2 NR | 7 V 2 | HN |
| 4 | 2 NR | 7 V 2 | HN |
| 5 | 2 NR | 11 | HN |
| 6 | 2 NR | 11 | HN |
| 7 | 2 NR | 11 | HN |
| 8 | 2 R | 7 V 2 | HN |
| 9 | 2 R | 11 | HN |
| 10 | 2 NR | 11 | HN |
| 11 | 2 NR | 7 V 2 | HN |
| 12 | 2 NR | V 3 V V 2 | HN |
| 13 | 2 NR | 7 V 2 | HN |
| 14 | 2 R | 11 | HN |
| 15 | 2 R | 11 | HN |

$k = 0.86$

| Observer | Facial Spots | Stripes Pattern | Horns State |
|---|---|---|---|
| 1 | 2 NR | 11 | HN |
| 2 | 2 NR | 11 | HN |
| 3 | 2 NR | 11 | HN |
| 4 | 2 NR | 11 | HN |
| 5 | 2 R | 11 | HN |
| 6 | 2 NR | 11 | HN |
| 7 | 2 NR | 11 | HN |
| 8 | 2 NR | 11 | HN |
| 9 | 2 NR | 11 | HN |
| 10 | 2 NR | 11 | HN |
| 11 | 2 NR | 11 | HN |
| 12 | 2 NR | 11 | HN |
| 13 | 2 NR | 11 | HN |
| 14 | 2 NR | 11 | HN |
| 15 | 2 R | 9 V | HN |

$k = 0.48$

| Observer | Facial Spots | Stripes Pattern | Horns State |
|---|---|---|---|
| 1 | 2 NR | II 8 V | HB |
| 2 | 2 NR | V 10 | HB |
| 3 | 2 NR | V 8 V | HB |
| 4 | 2 NR | V 10 | HB |
| 5 | 2 NR | 12 | HB |
| 6 | 2 NR | V 10 | HB |
| 7 | 2 NR | V 8 V | HN |
| 8 | 2 NR | V 8 V | HB |
| 9 | 2 NR | V 10 | HB |
| 10 | 2 R | V 10 | HB |
| 11 | 2 NR | 12 | HB |
| 12 | 2 NR | V 8 II | HN |
| 13 | 2 NR | 12 | HB |
| 14 | 2 NR | V 10 | HN |
| 15 | 2 R | V 10 | HB |

$k = 0.56$

| Observer | Facial Spots | Stripes Pattern | Horns State |
|---|---|---|---|
| 1 | 2 NR | 6 V 4 | HN |
| 2 | 2 NR | V 10 | HN |
| 3 | 2 NR | V 4 V 4 | HN |
| 4 | 2 NR | V 4 V 4 | HN |
| 5 | 2 R | 6 III 3 | HN |
| 6 | 2 NR | 7 V 3 | HN |
| 7 | 2 NR | 6 III 3 | HN |
| 8 | 2 NR | 6 V 4 | HN |
| 9 | 2 NR | V 3 V 5 | HN |
| 10 | 2 NR | 6 II 4 | HN |
| 11 | 2 NR | 12 | HN |
| 12 | 2 NR | V 4 III 3 | HN |
| 13 | 2 NR | 7 V 3 | HN |
| 14 | 2 NR | V 10 | HN |
| 15 | 2 NR | V 10 | HN |

*Figure 3: Results of Light's Kappa  in the IRR analysis. Codes given by the majority of observers are highlighted.*

## Discussion

Relying on a captive bongo population allowed the development of two tools that could prove helpful in the monitoring and conservation of this critically endangered antelope. Access to known individuals allowed the development and rigorous assessment of a visual ID-system, whereas access to areas exclusive for the target species allowed the recovery of reliable measures of their spoor. This allowed the retrieval of a measure (aspect ratio) which can help in assigning tracks to bongo, discerning these from similarly sized waterbuck.

Here, we used an information theoretic approach to develop a user based identification system to implement on camera trap footage. The use of three independent visual features (cheeks spots, horns and stripes) provides enough information to be effective in a population of 50 individuals. Hence, it responds to our need for an informative system appropriate for use in the field. One limitation in our assessment of the information content of the system is the assumption that the variation found in the captive population in MKWC is representative of the patterns found in the wild populations. Captive breeding, particularly domestication, is known to influence the appearance of a species (Snyder et al., 1996; O'Regan and Kitchener, 2005), however, the captive breeding of bongo has only lasted 40 years. Therefore, I consider valid the assumption that the variability seen in captivity is comparable to that in the wild.

Results from IRR analysis suggest that our ID system is reliable, with an overall substantial agreement amongst 15 naïve observers (kappa > 0.60). While these results are very promising, we would anticipate higher values of kappa with field-workers or those familiar with bongo footage. The system therefore responds to the need for a reliable tool to implement for long term monitoring with little or no need for experienced observers. Overall, the system presented here fulfils the need for an inexpensive, reliable, and readily adoptable tool for monitoring of wild bongos. The application of our ID-system to the photographic records collected by BSP would allow the transition from mere surveillance monitoring to detailed population monitoring using the same data and with no change needed in BSP monitoring routine.

Here, I used this approach for developing an ID-system that could help and reinforce the work of a Kenyan NGO in its effort of efficiently monitoring a critically endangered antelope. Nonetheless, any species with individual-specific visual features could benefit from a comparable ID-system. Our results show that even apparently uninformative features with little variation like cheek spots of bongo are useful in the identification process. Moreover, species with less clearly defined patterns could still benefit from the investigative component of my approach in defining the best variables for identification. So long as the camera trap footage involved reliably shows these features, the identification system can be used in historic footage collected without accommodating for a specific ID method. Reliance on a user-based identification system evaluated for reliability and information content could enhance and refine MR studies where automated software is not an option. This could be due to yet non-optimised software for the target species, or the lack of infrastructure for an efficient implementation of software-based tools. Our approach also alleviates the need for experienced observers to be ever-present in the monitoring program, thus increasing the likelihood that the monitoring can proceed in time, one of the assumptions for effective long-term monitoring (Legg and Nagy 2006; Lindenmayer and Likens 2009).

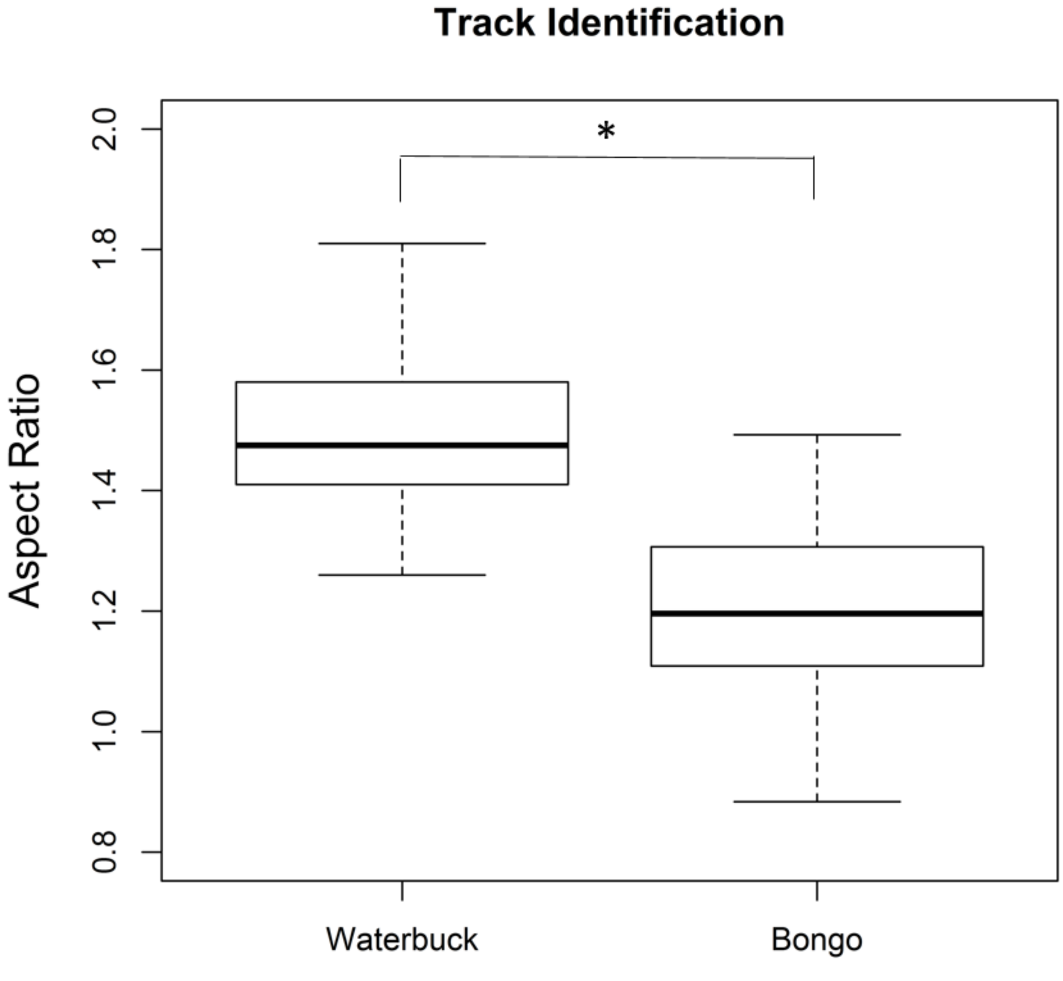


*Figure 4: Boxplot showing the difference in aspect ratio (length/width) of bongo and waterbuck tracks. Difference was found to be significant with a two sample t-test (t = 12.102, df = 148, p-value < 0.001)*

The finding of a measure (aspect ratio) which can help to assign tracks to bongo can be of great help to both practitioners and researchers involved with this antelope. The use of a quantitative method provides fieldworkers with a tool that can help in spoor identification, even in case of limited experience with the target species. Field workers can incorporate this measure with their expertise or that of their team when identifying tracks. This is particularly relevant for bongo, as local expertise is limited to a handful of former trackers (Prettejohn, 2008). For some values in my sample, there is an overlap between bongo and waterbuck (Figure 4). Nevertheless, the use of this variable as a predictor not only proved to be significant but also highly predictive (AUC > 0.8), and reliable (kappa > 0.6). Therefore, I would consider a safe approach to assign any track with an aspect ratio below 1.3 to bongo. Although no field validation was attempted for this study, in two occasions the author, when working with BSP trackers, was able to identify bongo tracks through their aspect ratio and, in both cases, the trackers had independently identified them as bongo. The intended use of this measure is to facilitate monitoring in areas where bongos are known to be present through camera trap records or previous research (Faria et al., 2011). Genetic barcoding of dung should be implemented whenever evidence of presence comes from novel areas. The approach here used to discern bongo and waterbuck tracks could also apply to dung, however, the free roaming nature of waterbuck in MKWC did not allow for an unambiguous retrieval of dung piles. During our study, only a single dung pile could be assigned to waterbuck with certainty. Moreover, differences in diet between captive and wild individuals may limit the reliability of such measure.

The access to a relatively large captive population of bongo was vital in obtaining the findings here presented. The development of an informative and reliable ID system would not have been possible without known individuals. Many endangered species are currently hosted in zoos and collections worldwide. While few have large enough populations to allow a replicate of our approach, images could be pooled from multiple institutions in order to increase sample size. The same can be said for spoor, as images rather than field measures could be used to try to find significant differences between similarly sized species. This further demonstrates the relevance of captive populations for the study of their wild counterpart, not only for their conservation value, but also because they can be a resource for developing tools and methods to then implement in the field, an integral part of the One Plan approach (Lees and Schwitzer, 2013).

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## Appendix I: Appendix 4 to National Recovery and Action Plan for the Mountain Bongo (*Tragelaphus eurycerus isaaci*) in Kenya (2019 – 2023)

# Appendix 4:

# Identification, monitoring, Body Scoring of Bongos

By: Tommaso Sandri, Fred Omengo, Bradley Cain, Martin Jones, Dave Mallon & Ed Harris

## Introduction

Protocol to aid in the conservation and Management of the Bongo:

i. A bespoke ID-System for bongo identification
ii. A quantitative method to identify bongo spoor in areas where waterbuck and bongo are sympatric
iii. Camera trapping protocol for monitoring
iv. The application of a pre-existing Body Condition Scoring (BCS) system to the captive bongo herd at MKWC

### I) ID-system

The lack of an identification system is a major impediment to the long-term monitoring of any animal population of conservation concern (Legg & Nagy 2006). Here we describe a user based visual ID-system that requires little training, and is fast and transferable.

The ID system was initially developed on the captive herd at the Mount Kenya Wildlife Conservancy (MKWC). The system relies on individual features of bongo flanks (Figure 1). Of these, stripe pattern have been found previously to be important for individual identification (Gibbon et al. 2015).

The system was tested for its transferability amongst multiple observers through the analysis of inter-relator reliability (irr, Hallgren 2012) using K statistics (Fleiss 1971, Landis & Koch 1977), where the closer the value of K to 1 the higher the agreement amongst observers. 15 naïve observers, who Ire neither trained in the system nor bongo experts, Ire asked to ID 10 bongo flanks. The results show a substantial agreement (average K = 0.65) amongst the 15 observers, thus showing that the ID-system here presented is transferable, reliable and can become a useful tool for long-term monitoring (Figure 2).

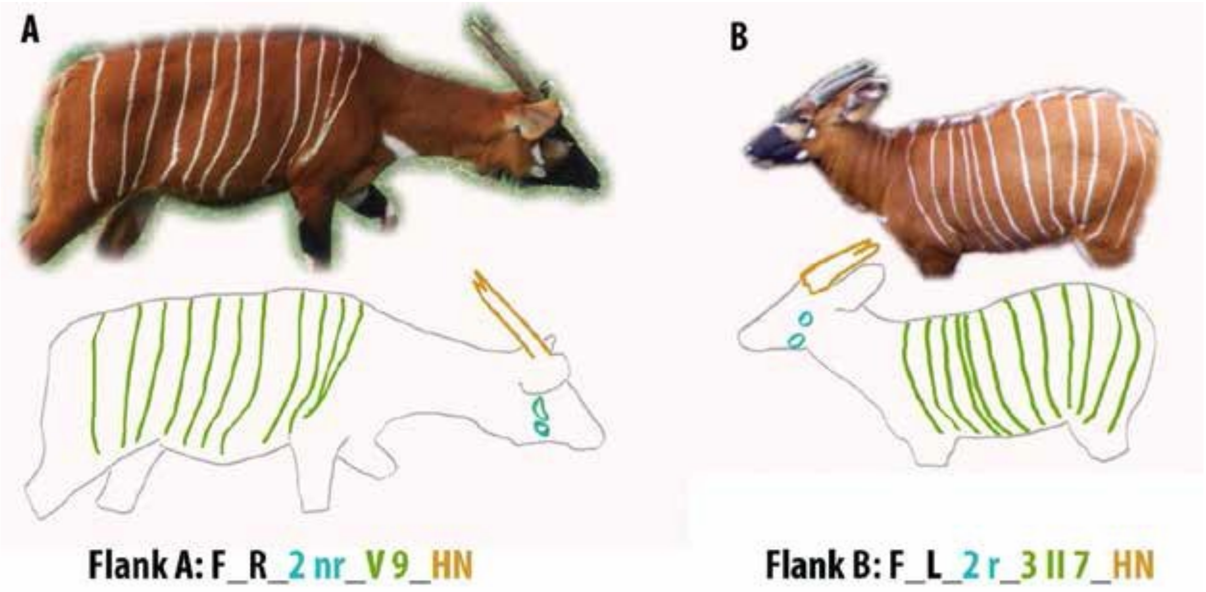


Figure 1: Example of our newly developed ID-system for bongo flanks: Flank A is coded as F (female), R (right flank), 2 nr (two facial spots, upper spot is not round), V (2 stripes converge), 9 (nine stripes with no peculiar feature), HN (horns appear normal). Flank B is coded as F (female), L (left flank) 2r (two facial spots, upper is round), 3 (three stripes with no peculiar feature), II (two stripes appear narrower than the others on the animal's flank), 7 (seven stripes with no peculiar feature), HN (horns appear normal).

Figure 2: BSP picture taken in the Salient (ANP) with individuals with the ID system code assigned by BSP.

**II) Track identification method**

The identification of bongo tracks is generally not problematic, however in areas where both bongo and the similarly sized waterbuck (Kobus ellipsiprymnus) are sympatric, misidentification can occur and waterbuck spoor can be wrongly identified as bongo (Faria et al. 2011). Misidentification of spoor can obviously have a significant impact on the reliability of any monitoring programme.

In an attempt to increase monitoring reliability, we have developed a quantitative method for distinguishing between the spoor of the two species. Thanks to the access to MKWC captive herd, we measured 100 bongo tracks and 50 waterbuck tracks. We opportunistically sampled and measured tracks in enclosed areas within the conservancy where only one of the target species was present. Our sampling did not differentiate among age-classes or sexes. Our results show that the length to width ratio (LW) averages 1.2 (± 0.15) for bongo and 1.5 (± 0.13) for waterbuck (Figure 3). A 2-sample t test found the difference to be significant ($p < 0.0001$). Subsequently, we included LW in a logistic regression (Dreiseitl & Ohno-Machado 2002) as a predictor of the species. Results show an AUC, a measure of predictive reliability of the logistic regression, of 0.90 out of a maximum value of 1.

The incorporation of two simple measurements easily retrievable in the field should greatly increase the reliability of bongo monitoring through spoor.

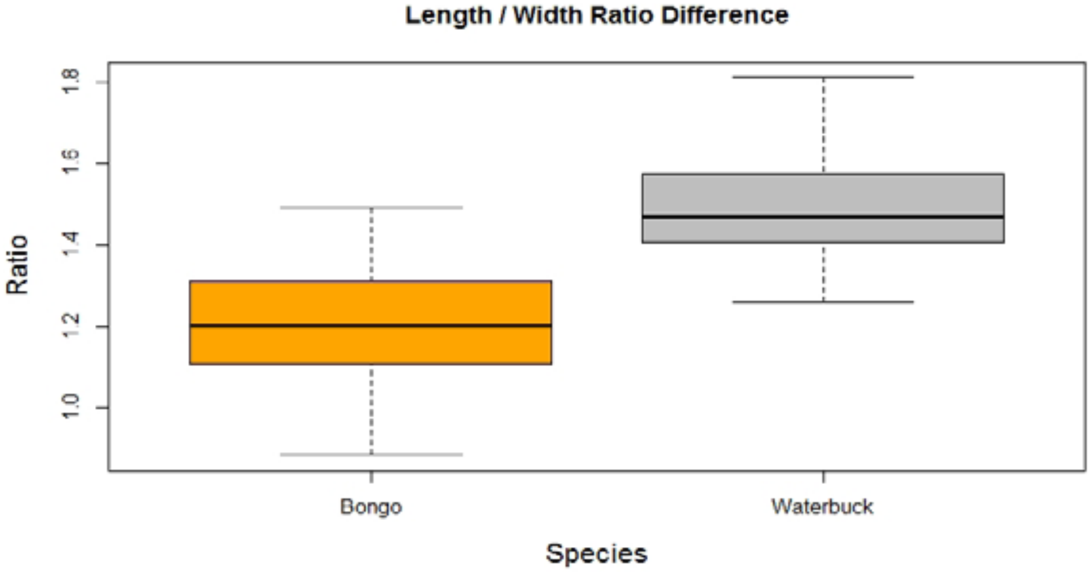


Figure 3: The plot shows the difference in length to width ration between bongo tracks and waterbuck tracks

### III) Camera trap protocol

The method is adopted from (O' Connel et al. 2011) on using camera traps to collect data for the development of a Habitat Suitability Model (HSM) for bongo. The use of both presence and absence points is considered the most accurate (Guillera-Arroita et al. 2015) method. In order to retrieve both presence and absences we implemented a random sampling covering the available habitat in the area of interest (Hirzel & Guisan 2002).

Cameras are placed at 1km from one another in a grid array. The devices are installed facing active game trails and tied on robust trees (to avoid interference from wind) and at a height of at least 1.5 m (to avoid disturbance from hyenas). Cameras are set to take 3 photographs per capture event during both day and night. Cameras are left in place for at least 10 nights.

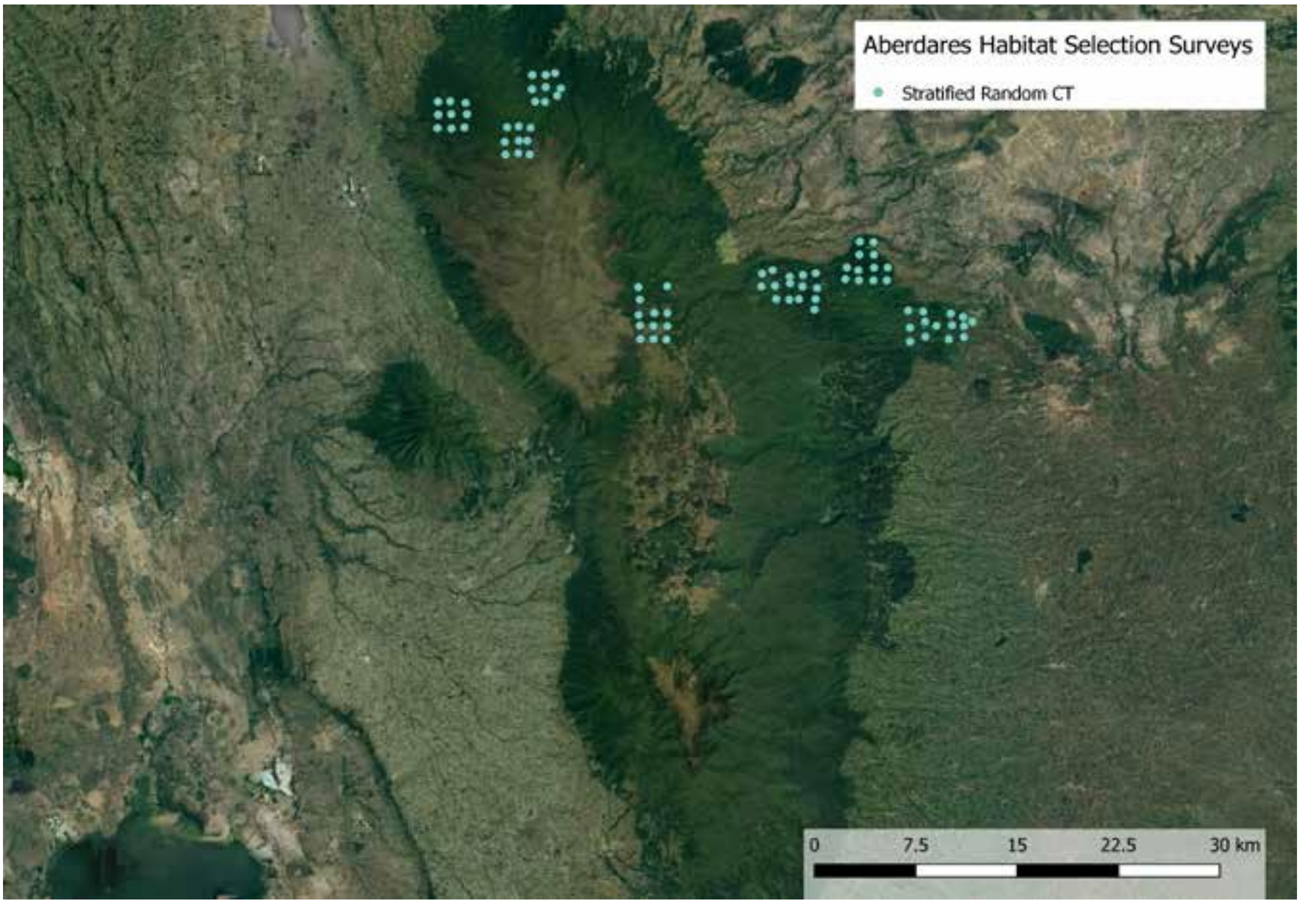


Figure 4: Map of bongo habitat selection survey sites in the Aberdare

Figure 5: A young male at the Salient (ANP, August 2018)

### IV) Body Condition Scoring System

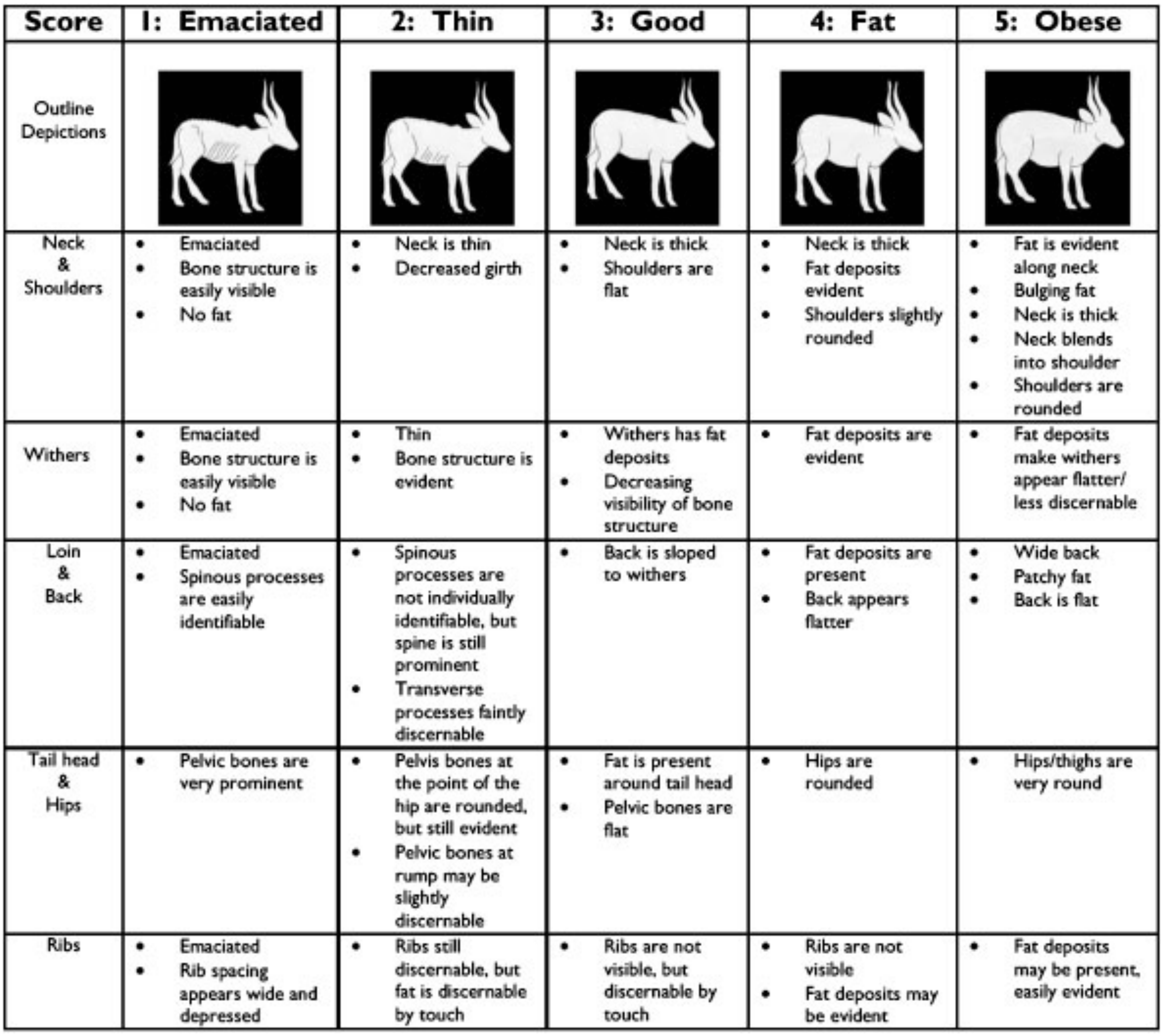
Assessing and evaluating the condition of individuals is of primary interest for conservation actions (Stevenson & Woods 2006). Captive individuals can be assessed for their suitability for release or breeding purposes and they can then be monitored after release using a standardised body condition system.

The use of a standardised system allows multiple practitioners to objectively evaluate the body condition of an individual animal. A standard system for monitoring body condition can be used to assess the welfare of captive individuals, which is relevant for their reproductive output. In order to assess the status of the bongo herd at MKWC we implemented a system previously designed for captive mountain bongo by Disney (Disney Animal Programs 2005, Figure 6). BCS scoring relies on visually estimating the amount of accumulated fat over various body parts (Wright et al. 2011).

The system was applied through photographic records of each individual rather than live encounter in order to test its applicability on pictures. This would allow the system to be remotely applied to individuals captured from camera traps. A mean body condition of 3.2 was obtained with the lowest score being 1 (found in one individual) and 4 (in 13 individuals) being the maximum (Figure 7 for examples). No individual was found to be obese (score 5). The scores appear comparable with results from a previous analysis in UK zoos (Wright et al. 2011).

Results from the captive herd were compared with wild individuals in the Salient area of the Aberdare NP. The wild individuals Ire scored using photographs retrieved from both MMU and BSP where the flank was clearly visible. The wild individuals mean BCS was 2.9 showing no significant difference to that of the captive MKWC population (Wilcoxon test: $P > 0.05$; Figure 8)

The application of an internationally recognised scoring system allows for the comparison of the MKWC herd with other institutions worldwide. Besides, the application of a standardised system will allow practitioners and managers to both evaluate individuals for their suitability for reintroduction and, when paired with a reliable ID-system, monitor individuals following the release.



| Score | 1: Emaciated | 2: Thin | 3: Good | 4: Fat | 5: Obese |
|---|---|---|---|---|---|
| Outline Depictions | | | | | |
| Neck & Shoulders | • Emaciated<br>• Bone structure is easily visible<br>• No fat | • Neck is thin<br>• Decreased girth | • Neck is thick<br>• Shoulders are flat | • Neck is thick<br>• Fat deposits evident<br>• Shoulders slightly rounded | • Fat is evident along neck<br>• Bulging fat<br>• Neck is thick<br>• Neck blends into shoulder<br>• Shoulders are rounded |
| Withers | • Emaciated<br>• Bone structure is easily visible<br>• No fat | • Thin<br>• Bone structure is evident | • Withers has fat deposits<br>• Decreasing visibility of bone structure | • Fat deposits are evident | • Fat deposits make withers appear flatter/ less discernable |
| Loin & Back | • Emaciated<br>• Spinous processes are easily identifiable | • Spinous processes are not individually identifiable, but spine is still prominent<br>• Transverse processes faintly discernable | • Back is sloped to withers | • Fat deposits are present<br>• Back appears flatter | • Wide back<br>• Patchy fat<br>• Back is flat |
| Tail head & Hips | • Pelvic bones are very prominent | • Pelvis bones at the point of the hip are rounded, but still evident<br>• Pelvic bones at rump may be slightly discernable | • Fat is present around tail head<br>• Pelvic bones are flat | • Hips are rounded | • Hips/thighs are very round |
| Ribs | • Emaciated<br>• Rib spacing appears wide and depressed | • Ribs still discernable, but fat is discernable by touch | • Ribs are not visible, but discernable by touch | • Ribs are not visible<br>• Fat deposits may be evident | • Fat deposits may be present, easily evident |

Figure 6: Bongo Body Condition Scoring System (Disney Animal Programs, 2005), figure from Wright et al. 2011.

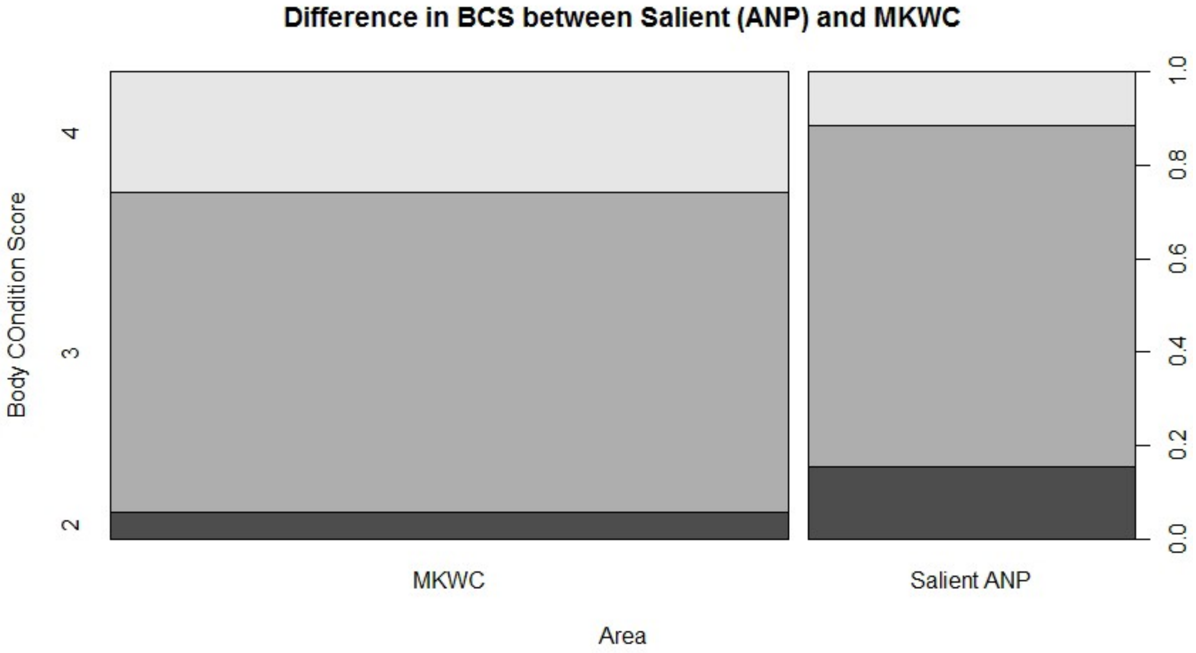


Figure 7: Body Condition Score in the Salient (ANP) and in captivity (MKWC)

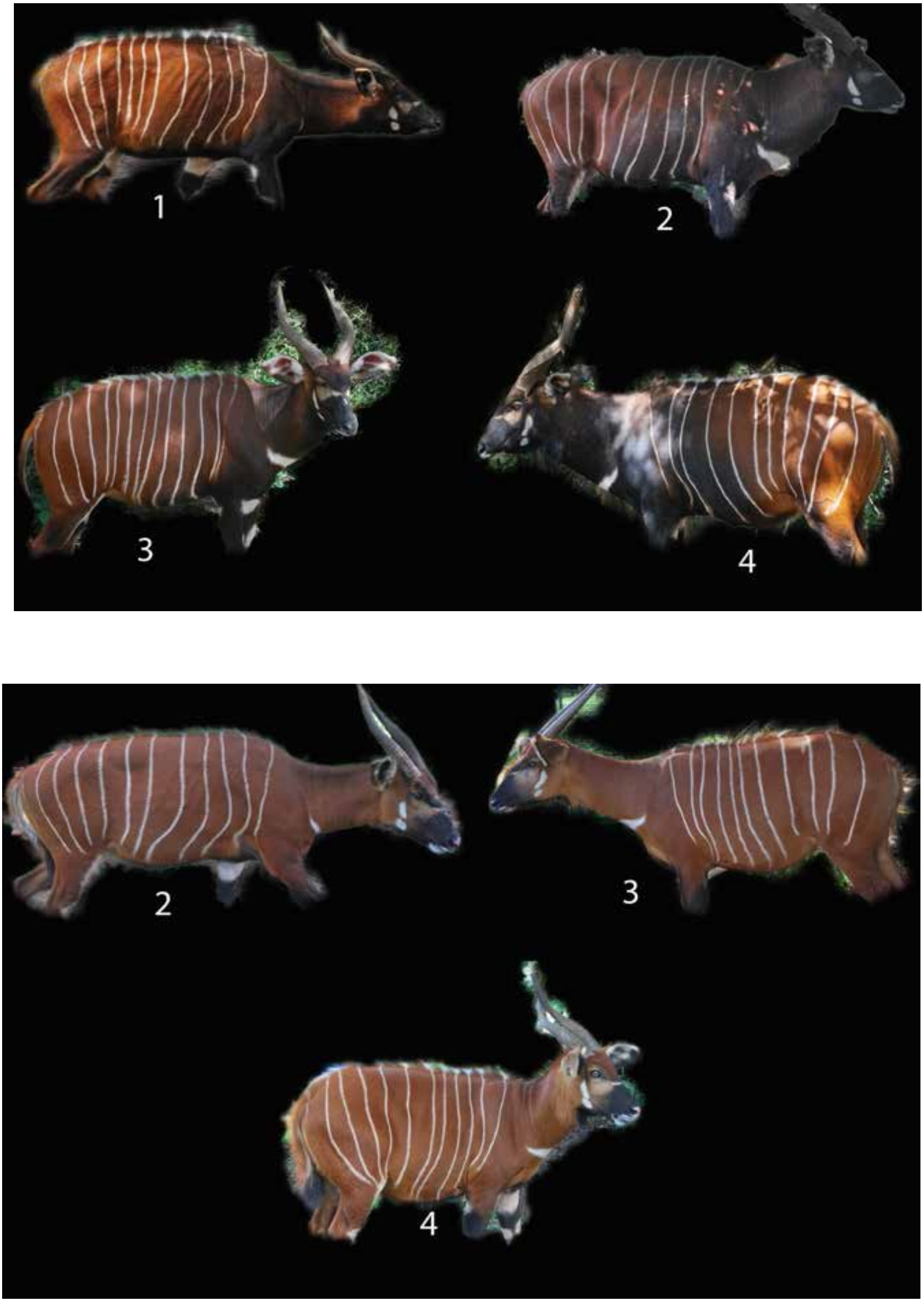


Figure 8: The image shows examples of bongo individuals from MKWC captive herd with relative body score assigned following the Disney scoring system.

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# Chapter 3:
# Population Monitoring of a Critically Endangered Antelope, the Mountain Bongo.

### *Context in the Thesis*

*Here, I implement the ID system presented in Chapter 2 on the extensive camera trap footage gathered by the Bongo Surveillance Project between 2013 and 2018. By conducting Mark-Recapture analyses I obtain information on the size, structure and dynamics of the four remnant populations.*

### Abstract

Monitoring is paramount for the gathering of valuable information on endangered species and populations in need of conservation actions. However, monitoring of a critically endangered antelope endemic to Kenya, the mountain bongo (*Tragelaphus eurycerus isaaci)*, has hitherto been limited to surveillance, thus gathering limited information. Here, I implement Robust Design Mark – Recapture using a newly developed identification system on existing camera trap footage to conduct population monitoring of the four remnant wild populations. I provide information on sex and age specific vital rates and the first estimates for bongo population size in the wild. Results show that only two of the four monitored populations include both sexes and calves, and these two populations have grown within the period covered (2013 – 2018). Moreover, survivorship appears to be influenced by sex and age, with males and calves suffering a higher mortality than females. Combined estimates of size of the two breeding populations of 39.58 (CI 29.2 – 49.8) confirm the critical situation of this antelope. My findings provide the first robust estimates of both vital rates, which will help in assessing their viability, and size and trend of the last surviving populations for mountain bongo, hitherto missing information which will help in its long-term conservation.

## Introduction

Species-focussed conservation aims to limit local and global extinction by designing and implementing actions for species protection and recovery (Hoffmann et al., 2010; Hoban and Vernesi, 2012), and in order to design effective actions, conservationist often rely on monitoring (Nichols and Williams, 2006; Clutton-Brock and Sheldon, 2010). Monitoring allows the retrieval of information on the persistence of populations, so called surveillance monitoring, or it can provide detailed information regarding dynamics with population monitoring (Nichols and Williams, 2006). Moreover, population monitoring provides information and estimates for vital rates (survivorship, migration rate, and fecundity among others), which can be used to assess the viability of the population in the long or short term (Johnson et al., 2010; Ali et al., 2018; Lacy, 2019). Thus, monitoring provides conservationists with fundamental information for implementing management strategies to promote recovery of endangered species (Yoccoz et al., 2001; McCarthy and Possingham, 2007; Lindenmayer and Likens, 2010).

A common monitoring method is Mark-Recapture (MR), which allows the retrieval of vital parameters and estimates of population size through subsequent encounters of marked or identified individuals (Lebreton et al., 1992). However, encountering individuals and marking or identifying them is challenging in areas with closed vegetation or difficult terrain such as forests (Rovero et al., 2014; Amin et al., 2016). The use of indirect approaches can alleviate some of the logistical challenges, and camera trapping has become a valuable tool when studying species in difficult areas (O' Connel et al., 2011).

Conservation of forest antelopes have benefitted from the use of camera trapping (Amin et al., 2016; Gray, 2018), as, due to their elusive nature and dense habitat, direct methods are unsuited for data collection (Bowkett et al., 2008). Relevant information like abundance, can be derived from density estimates without MR methods that necessitate individual identification, such as distance sampling (Thomas et al., 2010) and random encounter model (Rowcliffe et al., 2008). However, in order to retrieve indications on survivorship and other vital rates, monitoring relies on MR, and the need for individual identification has led to few, if any, studies on forest antelope vital rates. Lack of such information can hamper conservation actions (Milner-Gulland and Singh, 2016). Even more so when the need is to monitor a reintroduced population, and evaluate its viability. Therefore, MR and assessment of vital rates would greatly help conservation of endangered forest antelopes.

The mountain bongo (Tragelaphus eurycerus isaaci, hereafter bongo) is a critically endangered forest antelope endemic to the highland forests of Kenya. Global population has declined in the last century due to habitat loss, hunting pressure and disease outbreaks and its current range is limited to four isolated mountain areas in central Kenya: Maasai Mau, Eburu, Aberdares, and Mt Kenya (Faria et al., 2011). Although no precise abundance estimates exist, less than 100 are thought to remain in the wild, thus the listing as critically endangered (IUCN SSC Antelope Specialist Group, 2016). Lack of information regarding the remnant populations is mainly due to bongo inhabiting difficult terrain in montane forests combined with its elusiveness, which make direct sightings rare. Hence, although bongo have long been a focus of international conservation efforts, with particular emphasis on repatriation projects (Reillo, 2002; Veasey, 2010), information on its vital rates and a precise estimate of how many remain in the wild is hitherto missing.

To date, monitoring of in situ bongo remnant populations has relied on monitoring their persistence in time, by assessing the presence of animals without detailed information on population size and structure (i.e. surveillance monitoring). A local NGO, the Bongo Surveillance Project (BSP, www.mountainbongo.org), conducts monitoring of the remnant populations via camera trapping since early 2000’s (Prettejohn, 2004, 2008). Nonetheless, no attempt to obtain further information on population status and dynamics, which would provide quantitative information on the presence of individuals, was possible due to the lack of a reliable identification system. However, the implementation of a newly developed visual individual identification system for bongo (Chapter 2) would allow the retrieval of more information from BSP extensive footage obtained through their monitoring activity. Implementing a population monitoring system by combining an ID system (Chapter 2) using BSP extensive footage would allow the estimate of survivorship, thus providing conservation managers with important information that directly impacts population growth and, important for a critically endangered species, viability (Mills, 2013).

Intrinsic factors like age and sex can influence survivorship and cause intraspecific variations. Hence, in order to be effective, managers and conservationists need to assess the relevance of sex and age in populations of interest (Gordon et al., 2004). This is particularly relevant for large herbivores, where differences in survival amongst age classes affect population dynamics (Gaillard et al., 1998). Age is known to have the largest influence on survival in large herbivores, and recruitment of young individuals in the mature classes is the main limiting factor to population growth both in temperate and African ungulates (Gaillard et al., 1998, Owen-Smith and Mason, 2005). Further understanding of what drives bongo population dynamics can be achieved by assessing whether sex has an influence on the mortality of individuals. Previous research on greater kudu (*Tragelaphus strepsiceros*, a bongo relative) has shown that adult males suffer higher mortality than females, likely due to their larger size and the corresponding higher energetic demands (Owen-Smith, 1993). Therefore, understanding the influence of age and sex on bongo survivorship is paramount for effective management.

Size of the remnant populations (hereafter abundance), can also be retrieved through MR, and it would provide a benchmark of status of these populations, and therefore inform managers on the need and type of conservation actions (Yoccoz et al., 2001). Furthermore, using BSP monitoring data, abundance can be estimated for each year covered by BSP. This would provide a valuable indication of the wild bongo population trend. Results can indicate the effectiveness of ongoing conservation actions or the need for management change (Nichols and Williams, 2006), particularly effective if included in an adaptive management framework (Lindenmayer and Likens, 2009). Assessing survivorship in bongo, and the influence age class and sexes may have, will assist managers and conservationists investigate the chances of its recovery in the wild. Moreover, I will generate abundance estimates for the period covered by BSP work and evaluate the trends of the four remnant populations, a vital and hitherto missing information for the conservation of this iconic forest antelope. My work could help in the study of vital rates with MR in forest ungulates.

Thus, I will assess vital parameters and estimate abundance and trend in the remnant populations of this critically endangered antelope. I will i) identify individuals in footage collected by BSP, ii) conduct MR to retrieve vital parameters and model the influence sex and age may have, and iii) retrieve the first estimates for population size and the trend of the remaining wild populations. I discuss my results in the context of providing valuable information for the conservation of elusive and endangered species using MR.

## Methods

### *BSP Footage*

Camera trap footage collected by BSP between 2013 and 2018 was analysed. The footage results from 81 discrete surveys conducted in the four areas known to host bongo: Aberdares (N surveys = 24), Mt Kenya (N=22), Maasai Mau (N=19), and Eburu (N=13), are described in Table 1. Each discrete survey lasts three to four weeks with one to three cameras placed at salt licks known to be frequented by bongo through indirect signs such as dung and spoor. BSP monitors two salt licks in the Aberdares and one in the other areas using Bushnell Natureview HD and Bushnell Essential HD cameras. Cameras are set to take three photographs per capture, are active for 24 hours and rely on infrared flash for nighttime captures. Figure 1 shows the location of BSP camera traps. BSP surveys and the resulting footage only pertain to those areas where bongo herds have been located; hence, other areas may host uncontacted herds. Nevertheless, BSP has surveyed additional areas to the ones here mentioned, including other parts of the Mau forest and the northern Aberdares, without encountering any bongo herds.

### *Individual Identification*

I used a visual ID system to identify individual bongo (Chapter 2). Bongo are asymmetrical in their pattern, thus, due to non-paired camera trap design, it was not possible to match left and right flanks of individual animals. Hence, the flank rather than the individuals would be the subject of my individual capture histories, where the first capture counts as marking and further detections are recaptures. I split the data in two capture histories in each area: one for left flanks and one for right flanks and analysed separately (Wang & Macdonald 2009). I excluded flanks encountered once from the analysis as they could result from misidentification and bias survival estimates (Morrison et al., 2011).

*Table 1: Primary Occasion with Mid-Point and number of BSP surveys conducted. The interval (in years) between each Primary occasion is also shown.*

| Primary Occasion | Mid-Point (N surveys) | Interval in Years |
|---|---|---|
| **Aberdares** | | |
| 1 | April 2013 (3) | |
| 2 | May 2014 (5) | 1.1 |
| 3 | March 2015 (3) | 0.9 |
| 4 | August 2016 (4) | 1.5 |
| 5 | October 2017 (3) | 1.2 |
| 6 | July 2018 (4) | 0.8 |
| **Maasai Mau** | | |
| 1 | February 2014 (2) | |
| 2 | May 2016 (2) | 1.3 |
| 3 | December 2016 (3) | 0.7 |
| 4 | August 2017 (4) | 0.8 |
| 5 | August 2018 (3) | 1 |

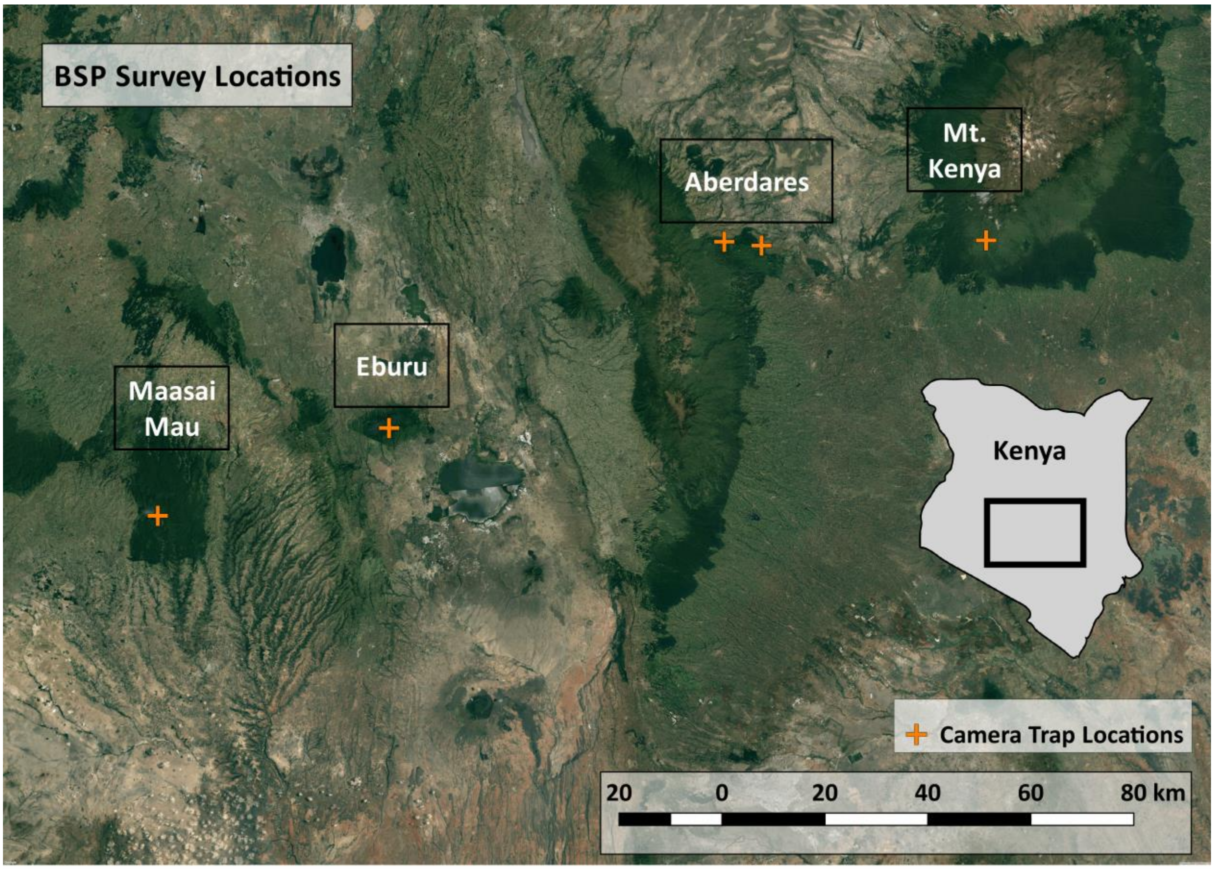

*Figure 1: The map shows the locations of BSP surveys.*

Bongo are characterised by sexual dimorphisms in both size and appearance. Females weigh between 250 – 300 kg while males can exceed 400 kg. Both sexes bear horns, but these grow larger in males, which are also characterised by a darker coat coloration when mature (Elkan and Smith, 2013). Bongo age, recorded as the age at first capture (i.e. age at marking), was estimated according to horn development, a method also used with other antelopes (Owen-Smith 1993; Marshal 2017). I implemented two age classes (immature, < 2 years, and adult), following methods described by Pollock (1981). Adult flanks were sexed according to horn shape and coat colour (Elkan & Smith 2013; Castello' 2016), whereas immature individuals can be sexed by observing the orientation of growing horns (divergent in males, almost parallel in females).

### *Vital Parameters*

Capture histories were analysed using the Robust Design model in MARK (Pollock, 1982; Huggins, 1989; White and Burnham, 1999; Kendall, 2018) to estimate probabilities of survival (*S*), first detection and recapture (*p* and *c*), temporary emigration, and population size as a derived parameter. I considered each year between 2013 and 2018 as a primary occasion, with the exception of Maasai Mau, where no surveys were conducted in 2015, while each survey was considered a single secondary occasion. I tested the Robust Design assumption of population closure for each of the primary periods included in the analysis using the Stanley-Burnham test (Stanley and Burnham, 1999) in the program CloseTest (Stanley and Richards, 2005). Abundance estimates retrieved in primary occasions found to violate the closure assumption were ignored. Three BSP surveys from 2018 that failed to meet the assumption of open population between the latest primary occasions were excluded (2017 and 2018).

I generated 21 *a priori* models allowing all parameters to be dependent on primary occasion (i.e. time), sex, and age at marking. The influence of age at marking was assessed applying age models to the parameters (Pollock, 1981). The set of models were designed considering current knowledge on bongo sociality and ecology: females are highly social and live in herds with their youngsters, whereas mature males are solitary and only occasionally join herds of females for breeding purposes, without however coercing or limiting their movement (Estes, 1992; Elkan and Smith, 2013). Therefore, I model capture and migration parameters as being equal in adult females and immature individuals as these are known to move together in herds (Kingdon, 1982; Estes, 1992). I evaluated the goodness of fit (GOF) of the most general model (Anderson and Burnham, 2002), here the model allowing for full dependence on sex, time, and age, for the encounter history of each area. Following Kendall (2018), I assessed GOF using the bootstrap GOF function in MARK with 1000 iterations, after which I calculated the p-value of a model with a higher deviance than the original as a measure of model fit.

An information theoretic approach was followed to conduct model selection by relying on AICc (Burnham and Anderson, 2002). Only models within $\Delta$AICc $\leq 4$ were deemed informative (Anderson and Burnham, 2002), and both vital rates and abundance estimates were averaged amongst these.

### *Population Trend*

Abundance estimates were used to assess the trend of the surveyed populations between 2013 and 2018 by calculating yearly population growth through $\lambda$, calculated as $\hat{N}(t) / \hat{N}(t - 1)$, where $\hat{N}$ is the abundance estimate and $t$ is the primary occasion (Owen-Smith and Mason, 2005). I assessed population growth relying on the abundance estimate of either right or left flanks depending on the more numerous captured flanks. I also considered estimates precision in deciding which estimate to rely on for $\lambda$ calculation (i.e. lowest Standard Error, SE).

## **Results**

### *Individual Identification*

In total, 102 flanks were identified in the footage spanning 2013 – 2018 in the four areas of interest, of these, eight were encountered only once. I therefore excluded them from the capture histories and MR analysis. A single flank was identified in both Mt Kenya and Eburu, a female and a male respectively. Therefore, no capture history was compiled and these areas were excluded from further MR analysis. Hence, MR analysis was conducted on the Aberdares and Maasai Mau populations, where the capture histories of identified flanks include both sexes and both age classes, as shown in Table 2. However, due to the limited size of the right flank sample in Maasai Mau, I used solely data from the left flanks.

*Table 2: Number of flanks (left:right) encountered in each period. Single captures are not presented.*

| | Aberdares | | | | |
|---|---|---|---|---|---|
| | Females | | Males | | Total |
| Period | mature | immature | mature | immature | |
| 1 | 5:5 | 1:4 | 3:2 | 1:1 | 11:12 |
| 2 | 5:5 | 2:5 | 2:3 | 3:3 | 12:17 |
| 3 | 5:5 | 1:5 | 3:1 | 3:2 | 12:13 |
| 4 | 6:6 | 4:7 | 2:2 | 2:6 | 17:21 |
| 5 | 7:5 | 6:6 | 1:1 | 4:3 | 18:15 |
| 6 | 7:7 | 6:9 | 1:1 | 4:5 | 18:22 |
| | Maasai Mau | | | | |
| | Females | | Males | | Total |
| | mature | immature | mature | immature | |
| 1 | 4:3 | 3:0 | 1:2 | 0:0 | 8:5 |
| 2 | 5:3 | 1:0 | 1:1 | 1:1 | 8:5 |
| 3 | 6:2 | 3:4 | 2:2 | 2:1 | 13:9 |
| 4 | 6:4 | 6:4 | 4:3 | 1:1 | 17:12 |
| 5 | 4:3 | 7:1 | 3:1 | 0:0 | 14:5 |

### *Vital Parameters*

The six most informative models (AICc ≤ 4, Table 3) indicate that both sex and age at marking influence survivorship, temporary emigration, and capture probabilities in bongo. Only one of the models (model 6, Table 3) shows no influence of either sex or age in the capture probability. All parameters were constant across the period of interest (2013 – 2018). Estimates for each vital parameter were averaged across the best models for each area using model averaging functions in MARK. Estimates for each area and each age and sex class are presented in Table 4.

*Table 3: Parameters estimates. Estimates are averaged among the most informative models (Δ AICC ≤ 4)*

| Parameter | Estimate (SE) | | |
|---|---|---|---|
| | *Salient Left* | *Salient Right* | *MM left* |
| Survival (*S*) AF | 0.99 (0.02) | 1 (0) | 0.99 (0.02) |
| Survival (*S*) IM & IF | 0.79 (0.12) | 0.99 (0.03) | 0.89 (0.11) |
| Survival (*S*) AM | 0.90 (0.11) | 0.89 (0.09) | 0.92 (0.14) |
| Temporary Emigration (γ'') AF & IF & IM | 0 (0) | 0 (0) | 0.19 (0.08) |
| Temporary Emigration (γ'') AM | 0.18 (0.20) | 0.11 (0.19) | 0.23 (0.12) |
| Capture Probability (*p*) AF & IF & IM | 0.51 (0.04) | 0.45 (0.03) | 0.55 (0.05) |
| Capture Probability (*p*) AM | 0.32 (0.06) | 0.33 (0.05) | 0.57 (0.08) |

*Table 4: Most informative (Δ AICC ≤ 4) of the 20 a priori models run in MARK. Resulting rank and AICc values for each capture history analysed are presented in the bottom half of the table.*

| Model ID | Model | N of Parameters |
|---|---|---|
| 1 | *S* (sex*age), γ''=γ' (.), p=c (sex*age) | 6 |
| 2 | *S* (age), γ''=γ' (sex/age(M)), p=c (sex*age) | 6 |
| 3 | *S* (sex*age), γ''=γ' (sex/age(M)), p=c (sex*age) | 7 |
| 4 | *S* (age), γ''=γ' (.), p=c (sex*age) | 5 |
| 5 | *S* (sex), γ''=γ' (sex/age(M)), p=c (sex*age) | 5 |
| 6 | *S* (sex*age), γ''=γ' (.), p=c (.) | 5 |

| **Aberdares** | | | | | | **Maasai Mau** | | |
|---|---|---|---|---|---|---|---|---|
| *Left* | | | *Right* | | | *Left* | | |
| Model ID | AICc | ΔAICc | Model ID | AICc | ΔAICc | Model ID | AICc | ΔAICc |
| 1 | 511.54 | 0.00 | 5 | 585.44 | 0.00 | 6 | 309.89 | 0.00 |
| 2 | 511.62 | 0.08 | 2 | 587.55 | 2.11 | 4 | 311.02 | 1.12 |
| 3 | 512.04 | 0.50 | 1 | 588.11 | 2.67 | 5 | 311.53 | 1.64 |
| 4 | 515.01 | 3.47 | 3 | 588.46 | 3.02 | 2 | 311.97 | 2.08 |
| 5 | 515.61 | 4.07 | 6 | 589.48 | 4.04 | 1 | 312.08 | 2.19 |
| | - | - | - | - | - | 3 | 314.18 | 4.29 |

*S: apparent survival*

*γ''=γ': random movement*

*(.): model with no sex or age dependence*

*(sex): the parameter is modelled as sex dependent*

*(age): the parameter is modelled as age dependent*

*(sex*age): the parameter is modelled as dependent on both sex and age*

*(sex/age(M)): the parameter is dependent on sex for all groups, only on age for males.*

*(p=c): capture probability equals recapture probability*

### *Population Trend*

Abundance estimates for Aberdares and Maasai Mau are presented in Table 5. The largest population is in the Aberdares and was estimated to have 23.11 (C.I. 17.11 - 29.10) individuals, and 16.47 (12.16 - 20.78) estimated in Maasai Mau. When estimating abundance, the capture history of left flanks from the first survey period in the Aberdares was found to violate the assumption of closure when tested with the Stanley-Burnham test ($p < 0.05$; Stanley and Burnham, 1999). Capture histories from Maasai Mau for primary occasions 1 and 2 both consist of two capture occasions, which is not enough for closure test. Hence, I ignored abundance results from these primary occasions, and I excluderespected the population closure assumption. Population abundance estimates for each primary occasion and the sex ratio of individuals encountered in Aberdares and Maasai Mau are shown in Table 5. Population growth was calculated as yearly λ relying on the abundance estimates, results are included in Table 5. Both populations have grown in the period of interest (2013 – 2018, Figure 2), although estimates for Maasai Mau are limited to the latest primary occasions (2016 – 2018, Figure 2).

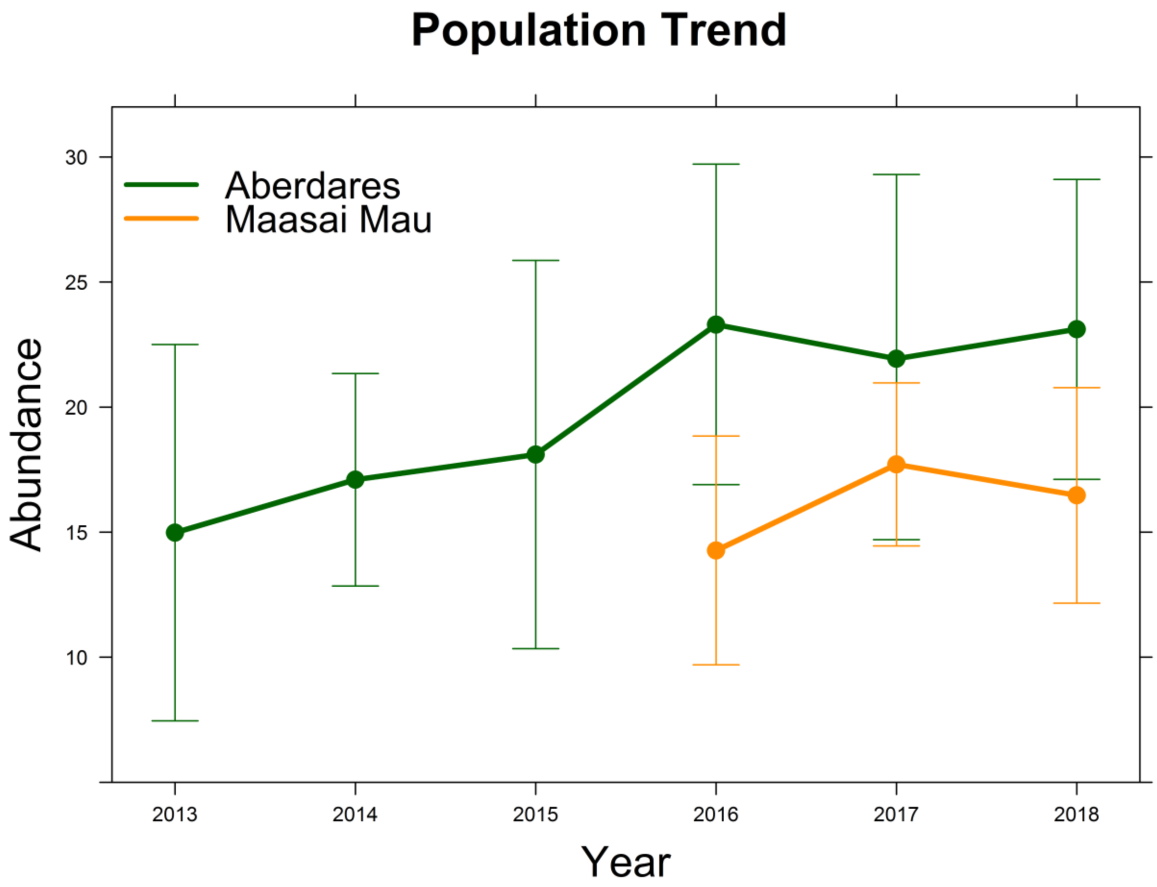

*Figure 2: Population trends in Maasai Mau and Aberdares, estimates are shown with 95% Confidence Intervals.*

**Discussion**

The combination of camera trapping on salt licks by BSP, my ID-system (Chapter 2), and MR has allowed me to assess population dynamics and vital rates of this critically endangered antelope, a task otherwise impossible to achieve in the forests of central Kenya. Salt licks are known to be relevant for bongo ecology (Klaus et al., 1998; Klaus-Hügi et al., 2000), and the approach of relying on these locations to study bongo population has been used in the past (Hillman, 1986). The same approach has been successful in studying populations of non-ungulates too (Galvis et al., 2014), where its accuracy was comparable to that of direct sightings along transects. A limitation of the approach here followed is the impossibility to include a spatial component in the analysis (Royle, 2015), which would be of great help in defining home ranges of herds and individuals, specifically males. Nevertheless, forest antelopes are generally difficult to study and monitor in their habitat, due to difficult terrain and their elusiveness (Amin et al., 2016). Moreover, when camera traps are deployed in an array individual identification can be challenging, due to the different angle at which an animal may approach the camera, whereas at salt licks flank are easily visible and identifiable. Identification at licks is easier due to the higher number of pictures that ca be retrieved of every single animal at multiple angles, in particular full profile view. Therefore, a solution to expand current monitoring efforts without losing identifiability of individuals may consist in creating an array of monitored licks, which would help in refining the area used by a particular herd, as from previous study (Klaus-Hügi et al., 2000) it appears bongo rely on, and visit, multiple licks. Nevertheless, the work here presented opens the possibility of providing future conservation actions, such as reintroduction of individuals from captive breeding, a framework for successful and effective monitoring of newly established populations, while causing minimal disturbance. The use of camera traps placed at salt licks, paired with a visual ID system could be enhanced by the use of marking techniques on individuals set to be released (ear notches or tags) which would further alleviate the issue of misidentification, thus allowing an even more rigorous monitoring. Moreover, the use of regularly visited sites can help conservationists and managers in successfully monitoring other wild forest ungulates, which are otherwise difficult to encounter and study.

All the best models resulting from our analysis in MARK include either sex or age, or both, as relevant in influencing survival in bongo (Table 3). Difference in survival of males compared to females appears to be typical of the *Tragelaphus* genus. Owen-Smith (1993) recorded a lower survival in male Greater Kudu (*Tragelaphus strepsiceros*), while Marshal (2017) found the same pattern in Nyala (*Tragelaphus angasi*). A common explanation for this difference in ungulates is the higher energetic demands of larger males compared to females (Bowyer, 2004). Moreover, the solitary lifestyle of adult males can expose them to a higher risk of predation (Owen-Smith, 1993). Both these hypotheses can help in understanding the influence of sex on bongo survival: adult males are larger than females, and being solitary could expose them to a higher predation risk. This latter point is further confirmed by our finding that the probability of temporary leaving the sampled area (γ'') is higher in males, and their encounter probability (*p*) is lower, which I interpret as males being prone to roam a larger area than herds of females. The limited sample size excluded an analysis with more than two age-classes. However, I would expect non-fully grown males that have left the maternal herd to be more vulnerable, both due to predation and to the higher energetic costs encountered during dispersal (Benoit et al., 2019).

*Table 5: Encountered flanks and Abundance estimates for each period. The sex ratio of encountered mature individuals in each session and population growth are also presented.*

| Primary Occasion | Captured Flanks (left:right) | N̂ (C.I.) | Sex Ratio | λ |
|---|---|---|---|---|
| | | **Aberdares** | | |
| 1 | 11:12 | 14.98 (7.46 - 22.5) | 0.6 | 1.14 |
| 2 | 12:17 | 17.1 (12.85 - 21.34) | 0.45 | 1.06 |
| 3 | 12:13 | 18.1 (10.34 - 25.86) | 0.4 | 1.29 |
| 4 | 17:21 | 23.3 (16.9 - 29.71) | 0.5 | 0.94 |
| 5 | 18:15 | 21.93 (14.7 - 29.3) | 0.37 | 1.05 |
| 6 | 18:22 | 23.11 (17.11 - 29.1) | 0.43 | - |
| | | **Maasai Mau** | | |
| 1 | 8:5 | 8.74 (3.85 - 13.64) | 0.2 | - |
| 2 | 8:5 | 9.99 (4.18 - 15.81) | 0.2 | - |
| 3 | 13:9 | 14.27 (9.7 - 18.84) | 0.63 | 1.24 |
| 4 | 17:12 | 17.71 (14.45 - 20.97) | 0.41 | 0.93 |
| 5 | 14:5 | 16.47 (12.16 - 20.78) | 0.34 | - |

*Table 6: Yearly survival estimates (SE) for individuals in the Aberdares.*

| | Mature Females | | Mature Males | | Immature (Males & Females) | |
|---|---|---|---|---|---|---|
| | left | right | left | right | left | right |
| 1 | 1 (0) | 1 (0) | 1 (0) | 1 (0) | 1 (0) | 1 (0) |
| 2 | 1 (0) | 1 (0) | 1 (0) | 0.78 (0.23) | 0.5 (0.36) | 1 (0) |
| 3 | 1 (0) | 1 (0) | 0.7 (0.2) | 1 (0) | - | - |
| 4 | 1 (0) | 1 (0) | 0.75 (0.24) | 0.61 (0.23) | 0.89 (0.14) | 0.86 (0.16) |
| 5 | 0.97 (0.09) | 0.96 (0.1) | 0.71 (0.23) | 0.94 (0.28) | 0.53 (0.27) | 1 (0) |

The very high survival of adult females is likely due to the fact that even when not fully grown, they still reside in the maternal herd, which heightens protection from predation (Jarman, 1974). Although my sample size was limited, the lack of large predators may explain the overall high survival rates of both sexes, as these appear closer to the rates found in temperate ungulates (Gaillard et al., 2003), living in areas with no large predators, than those typical of large herbivores in African savannas (Owen-Smith and Mason, 2005). Nevertheless, predation may play a role in shaping survival of newborn calves and young individuals. However, the estimates for survival are high for individuals of this age-class encountered at salt licks surveyed by BSP (0.79 – 0.99, Table 4). This confirms the idea that the gregariousness of females likely protects calves from predators. However, calves encountered at licks may not represent all the calves of the year, this is due to the habit of bongo, and the majority of antelopes, to give birth and keep the young separated from the herd for a certain period. The mortality in this crucial period was not estimable in my study, and this may have a larger influence on population dynamics than the survival of older calves once they join maternal herds and start visiting licks (Hillman, 1986). I could have followed an alternative approach in assessing the mortality of newborn calves: the ratio between females and calves (Owen-Smith and Mason, 2005). This measure assumes that each female in a herd is breeding in a particular season and therefore every female without a calf at heel should be considered as a lost calf. However, this measure has been proven to cause biased estimates (Bonenfant et al., 2005).

Providing managers and practitioners with estimates of survivorship of this critically endangered antelope will allow for the analysis of the viability of these remnant populations, a paramount step in assessing the extinction risk of bongo in the wild. By including the estimates here presented, both for abundance and survivorship, in a viability model (e.g. vortex, Akçakaya and Sjoegren-Gulve, 2000), managers could assess which of the populations are likely to keep growing or decline in the future. This would lead to better management of the wild populations with a more data-driven and evidence based approach. Moreover, having managed to obtain differential estimates for the sexes will allow for more detailed, and rigorous, predictions. Furthermore, by assessing survivorship on a yearly basis, as shown in Table 6, it appears likely that the population in the Aberdares may have reached its carrying capacity, as highest mortalities follow years with highest growth. However, such a conclusion is drawn from a relative short monitoring period (~ 6 years) and should therefore be further assessed, by continuing the current monitoring. Nevertheless, the results here presented may provide a benchmark for the carrying capacity component in viability analyses.

In addition to vital rates estimates, this work also presents the first abundance estimates for wild bongo populations. The largest population, in the Aberdares, was estimated to have 23.11 (C.I. 17.11 - 29.10) individuals with 16.47 (12.16 - 20.78) estimated in Maasai Mau, for a combined estimate of less than 40 individuals. This is lower than reported by IUCN (2016), and further proves the critical situation of this antelope in the wild. While the largest population of the Aberdares resides entirely in a national park, and can therefore be considered fully protected, Maasai Mau area is not yet fully protected, and evidence of its importance for this iconic Kenyan endemic can help those advocating for the conservation of the Mau forest complex (Nkako et al., 2005). For Eburu and Mt Kenya I detected only a single individual each. These estimates will provide valuable information for managers of these wild populations, as knowledge on the situation of bongo in the wild was set as a priority in the recently published recovery and action plan (KWS, 2019). I also report on the structure of bongo populations, with the structure of Maasai Mau and Aberdares population was assessed for the first time through their sex ratio (Table 5). A skewed ratio in favour of females is typical of antelopes (Jarman, 1974). The sex ratio found in the Aberdare population is similar to that of other African antelopes where males are mostly solitary (Owen-Smith and Mason, 2005). However, considering the very small size of these populations, a skewed sex ratio may be problematic, as low number of males can lead to females failing to conceive in a season with an immediate impact on population dynamics (Milner-Gulland et al., 2003; J. Rankin and Kokko, 2007). Although no attempt at reconstructing population networks within the two areas, it appears that in both Maasai Mau and the Aberdares all individuals belong to a single herd, as the same individuals are encountered together, this likely indicates that the two known populations in the wild consist of only two herds.

However, there may be other herds beyond the ones here assessed in Maasai Mau and in the Salient area in the Aberdares. Due to their elusive nature bongo are difficult to locate, and the analysis here presented pertains to those populations located during the extensive work of BSP. Although BSP work has thus far failed to locate any other areas where bongo may persist, it should be noted that results from Habitat Suitability modelling highlight the presence of bongo habitat in the southern Aberdares, and other parts of the Mt. Kenya forest reserve (Chapter 4). Although bongo have not been sighted in these areas yet, it should be a priority to expand the current efforts in locating additional populations, even more so in light of the results here presented.

My results confirm the critical situation of mountain bongo in the wild, with less than 40 individuals remaining in two isolated populations. Although both populations appear to have grown in the period covered by BSP, the extremely low numbers call for conservation actions aimed at reinforcing wild populations, by establishing additional populations in suitable areas. Moreover, results here presented highlight the relevance of the Mau forest for the long-term conservation of this iconic antelope, a flagship species for the entire afro-montane ecosystem.

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# Chapter 4:
# The Development of a Species Distribution Model for the Critically Endangered Mountain Bongo in light of novel information on its demography.

### *Context in the Thesis*

*Considering the results from the Mark-Recapture analysis in Chapter 3, it is clear that breeding herds occupy only two of the four areas known to host bongo. Therefore, I develop a novel Species Distribution Model relying on presences located only in these areas where bongo populations are thriving. I predict habitat suitability throughout the known range in order to highlight areas of interest for future conservation actions.*

### Abstract

Information regarding the location of optimal habitat for endangered species is paramount in designing and implementing conservation actions. Although a species distribution model had already been developed in previous studies for the critically endangered mountain bongo (*Tragelaphus eurycerus isaaci*), recent findings on the demography of the remnant populations incites a novel analysis including solely the two areas hosting breeding populations (Maasai Mau and Aberdares in central Kenya). Thus, the resulting predictions would refer to habitat that is optimal for presence and persistence of this antelope. Following a presence – absence approach I implement both camera trapping and sign surveys to retrieve locations within the areas of interest. I use Random Forest to predict the location of optimal habitat throughout this antelope known range relying on remote sensing measures for both vegetation and topography. The resulting model is both accurate (kappa = 0.7) and powerful (AUC = 0.92), and it confirms the relevance of vegetation in shaping bongo distribution. Habitat suitability predictions across the range indicate that Maasai Mau, the southern Aberdares, and parts of Mount Kenya likely host optimal bongo habitat. Moreover, a brief analysis on the influence of human disturbance (here represented by selective logging) is in agreement with previous research on the

limited impact of selective and small-scale logging. The information here presented will help managers and conservationists in designing and implementing conservation actions aimed at the recovery of the wild bongo population, by allowing them to focus on areas where bongo are likely to thrive. These areas could be prioritised for protection, if they are already occupied by bongo herds, or become candidate areas for future reintroduction.

## Introduction

Species distribution modelling (SDM) allows for the analysis of species presence in association with environmental variables in order to spatially predict habitat suitability (Guisan and Zimmermann, 2000; Guisan and Thuiller, 2005; Elith and Leathwick, 2009, Weber et al., 2017). Hence, practitioners and managers can use results from SDMs to inform conservation actions (Guisan et al., 2013; Villero et al., 2017). This has made SDMs a valuable tool in conservation biology (Rodríguez et al., 2007).

Developing SDMs relies on occurrence data of, typically, a single target species (Rondinini et al. 2006). Museum collections or other databases containing occurrence locations can be used in a so-called presence-only approach (Yackulic et al. 2013). Alternatively, the inclusion of absence points, usually located through field surveys, allow using a presence-absence approach, which is preferable for developing predictive SDMs (Guillera-Arroita et al. 2015). Although the collection of presence-absence data is logistically cumbersome, the use of indirect approaches like camera trapping have facilitated researchers, allowing the sampling of remote areas with minimal effort, an important feature when working with elusive or rare species (O' Connel et al., 2011; Rovero et al., 2014). Thus, the use of camera trapping allows developing SDM following a presence-absence approach when working with rare and elusive species living in difficult terrain.

Remote Sensing (RS) approaches help in deriving measures of environmental features associated with occurrence data (He et al., 2015). The use of spectral indices derived from multi-spectral satellite imagery allows evaluating vegetation presence, structure, and productivity of an area of interest (Xue and Su, 2017). Other sensors allow the retrieval of elevation, from which one can derive measures of topography, like the Shuttle Radar Topography Mission (SRTM, http://srtm.usgs.gov). Hence, the growing popularity of RS tools in developing SDM (He et al., 2015). Moreover, due to their landscape nature, these RS measures allow to predict vegetation and topography throughout a certain area of interest, be it at the landscape or regional scale (Guisan et al., 2017).

SDMs are inherently scale dependant, as are the features assessed in their development (Bradter et al. 2013). At the regional level, climatic aspects might have more relevance than fine vegetation structure in a landscape, whereas when predicting suitable habitat at a landscape scale climate might be too coarse a feature to derive effective predictions (Guisan et al., 2017). In applying SDM for conservation of endangered species with a limited or fragmented range, a landscape scale allows precise evaluation of areas with suitable habitat.

The aim of SDM is to predict areas suitable for species habitation, which is, predicting areas where the species could persist and thrive (Weber et al., 2017). Therefore, a basic knowledge of the demography of the populations occupying an area can improve the likelihood that what the SDM is predicting is habitat suitable for a good population rather than for individuals at the margins of the species range (Guisan et al., 2017). Thus, by assessing habitat associations in areas occupied by source rather than sink populations (Battin, 2004), one can develop SDM that predict suitable areas that allow populations to persist and thrive, which is relevant when predictions are used to inform conservation actions such as reintroduction (Osborne and Seddon, 2012; Guisan et al., 2013).

Biotic and topographic features of an area may be optimal predictors of habitat suitability, but an abiotic factor that is more and more included as relevant when considering habitat for endangered species is anthropic interference and disturbance in the area (Brodie et al., 2015; Strindberg et al., 2018). Human disturbance (hereafter disturbance) has shown to affect both habitat selection and activity patterns of species (Cavada et al., 2016; Rich et al., 2016; Oberosler et al., 2017; Gaynor et al., 2018). Therefore, including measures of disturbance when assessing habitat associations would improve our insight into its influence on the suitability of an area for a species, a relevant aspect when working with endangered species with a limited range fragmented within a human dominated landscape (Guisan and Thuiller, 2005).

The mountain bongo (*Tragelaphus eurycerus isaaci*, hereafter bongo), is a large (males weigh up to 400 kg) forest dwelling antelope endemic to the highlands of central Kenya (Elkan and Smith, 2013). Bongo occurrence is limited to four isolated mountain areas: Maasai Mau, Eburu, Mt. Kenya, and Aberdares (Faria et al., 2011), which are surrounded by a mostly agricultural landscape (Gichuki, 2008; UNEP, 2012). This antelope is categorised as critically endangered (IUCN SSC Antelope Specialist Group, 2016) and recent estimates of wild population indicate that less than 50 individuals remain in the wild (Chapter 3), hence highlighting the critical situation of bongo remnant populations. With its limited and fragmented range, and the scarce numbers in remnant populations, the development of an SDM is relevant for the conservation of this antelope as, although restricted in range, a better understanding of where bongo habitat is located within these areas would help conservation actions. As bongo have long been the focus of population restoration plans (Reillo, 2002; Veasey, 2010), information on which of these areas appear to host most suitable habitat can direct future reintroduction effort. Thus, an SDM developed on areas where populations are breeding can indicate priority areas for long-term conservation of this antelope.

Previous research has shown that bongo are heavily reliant on structured vegetation in their habitat (Estes et al. 2008; Sandri 2014), vegetation composed of trees with a rich growth in the understorey comparable to that of a secondary forest (Chokkalingam and De Jong, 2001). Topography, particularly slope, is also relevant for bongo presence (Estes et al., 2011). Estes (2011) provided a rigorous and thorough SDM for bongo developed with sign surveys conducted in Aberdares and Mt Kenya. However, at the time no information on the status of these populations was available. Hence, it is possible that the SDM developed encompassed marginal or sink populations, not fully representative of suitable habitat for a thriving population. Moreover, this study only predicted suitable areas in the Aberdares and Mt Kenya, then thought to be the most important areas for bongo. Recent findings (Chapter 3) however, show that only two areas are home to breeding herds: Maasai Mau and Aberdares. These areas host populations that are growing, hence the need for a novel SDM reliant on these areas for predicting suitable habitat in the entire range of this antelope. A model developed relying on areas occupied by thriving populations would provide an effective tool in both driving actions aimed at preserving current remnant populations, and also direct future reintroduction projects.

Here, I develop an SDM for the critically endangered mountain bongo relying on extensive camera trapping and sign surveys. My objectives are: I) assess habitat associations through measures of vegetation and topography; II) provide a prediction of suitable habitat throughout the four mountain areas of central Kenya; III) assess whether human disturbance influences bongo habitat selection. I discuss my results in the context of using SDM for managing endangered species, with specific recommendation for bongo conservation.

## Methods

### *Study Area*

The current range of the mountain bongo encompasses four mountain areas in central Kenya (Faria et al., 2011). Of these, I focused on the only two home to breeding populations: Aberdares and Maasai Mau (Chapter 3). The Aberdares are a volcanic massif spanning an elevation of 2000 to 4000 meters (Estes et al., 2008). Vegetation is characterised by shrubland, open and closed canopy Afromontane forests dominated by *Podocarpus*, *Juniperus*, and *Cassipourea* (Estes et al., 2008) at lower to mid-elevations. Higher elevations are characterised by bamboo (*Sinarundinaria* and *Bambusa*), *Hagenia* forest and afroalpine moorland (Estes et al., 2008). The overall lower elevation and altitudinal gradient (2000 – 2500 m) in the Maasai Mau is reflected in a lower vegetation diversity, being mostly characterised by Afromontane forests (Nkako et al., 2005; Baldyga et al., 2008). These areas were sampled using two methods to generate presence-absence data: camera trap and sign surveys.

### *Camera Trapping*

Camera traps were deployed using randomly generated grids arbitrarily placed in different areas in order to cover both the elevational gradient and the range of habitats that the target species could feasibly occupy within the study areas (Hirzel and Guisan 2002). The girds covered the entire range of vegetation present within the Aberdares, Figure 1. This allowed the recovery of both presence and absence locations. Grids apices were 1x1km apart in order to assume independence of captures. Surveys varied in space and time as described in Table 1. The terrain in the Aberdares and Maasai Mau is difficult, with steep valleys, thick vegetation, and the presence of potentially dangerous animals like elephants (*Loxodonta africana*) and buffaloes (*Syncerus caffer*). In the Aberdares seven surveys were conducted and camera positioning had to be altered twice within 300m of grid apices due to steep terrain rendering dangerous the positioning at the randomly generated location. Whereas in Maasai Mau both thick vegetation and steep terrain forced the change of camera deployment from 1x1 km to 1 x 0.5 km grid. Due to homogeneity in habitat, I conducted a single survey in Maasai Mau with 12 cameras (Figure 2).

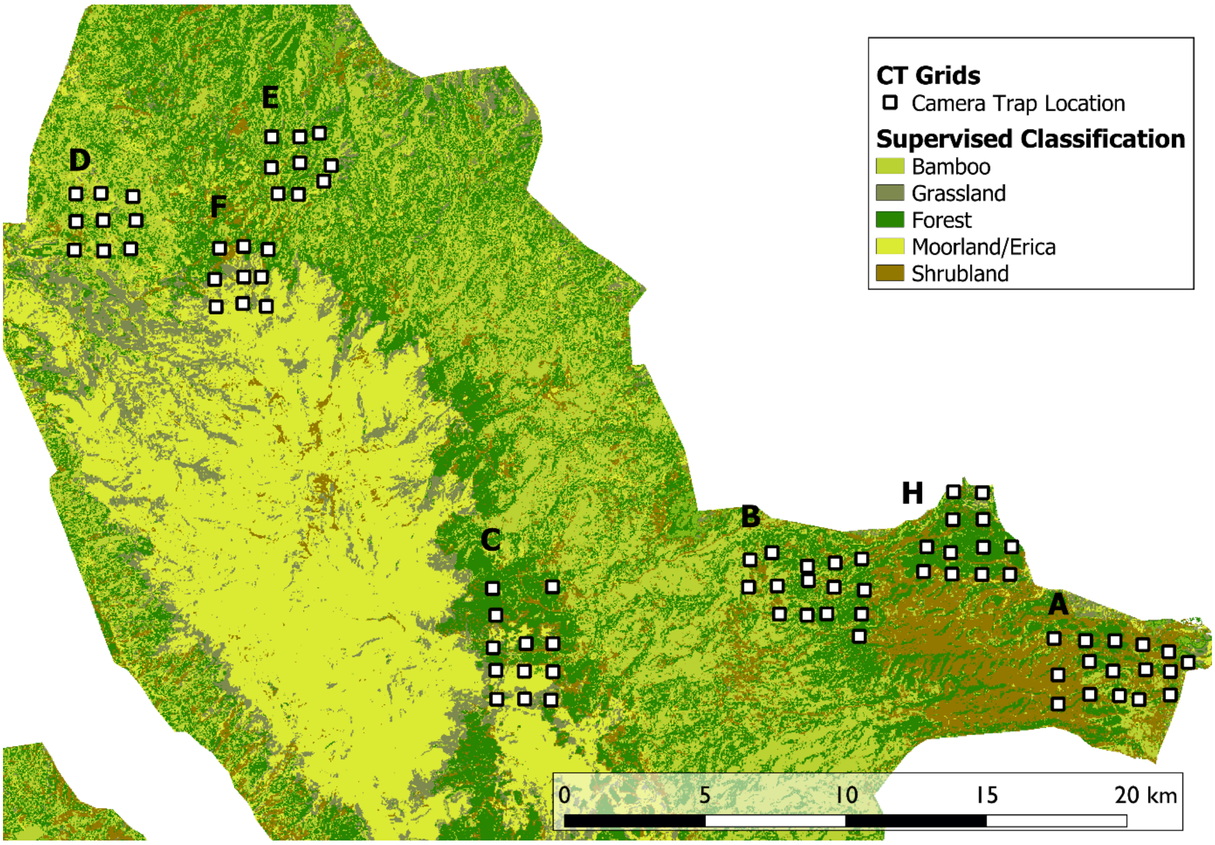

*Figure 1: Camera Trap locations in grid surveys conducted in the Aberdares. The map shows a vegetation supervised classification (from Morrison, 2019) showing the entire range of vegetation present was included in the surveys.*

Data were also collected using targeted location of known bongo presence resulting from surveys conducted in 2014 and 2016. These consisted of 9 to 12 cameras placed at no less than 30m apart in areas where the author or experienced rangers located signs of bongo presence (Figure 3). These surveys were of a “deliberately biased” nature, a technique to offset the low probability of detection for rare and elusive species (Meek et al., 2014), and their duration was limited to 6 days in order to allow the coverage of a multiple areas within the field-season. A third Camera trap survey was conducted in the southern area of the Aberdares in 2017 (Figure 4). This area is characterised by steep valleys with thick vegetation, mainly bamboo, therefore I excluded the use of random grids due to safety and logistical concerns. To survey this area I placed 9 cameras along four ridges, these are known by experienced trackers to be used by animals for roaming the area. Ridges are also the only way to access the mountain in the southern Aberdares. Along the ridges, cameras were placed in locations deemed as promising for bongo detection by a local experienced tracker and rangers. This survey was conducted in collaboration with Bongo Surveillance Project (BSP), with the aim to assess the presence of bongo population in the area based on historical range data (Prettejohn, 2008, 2011).

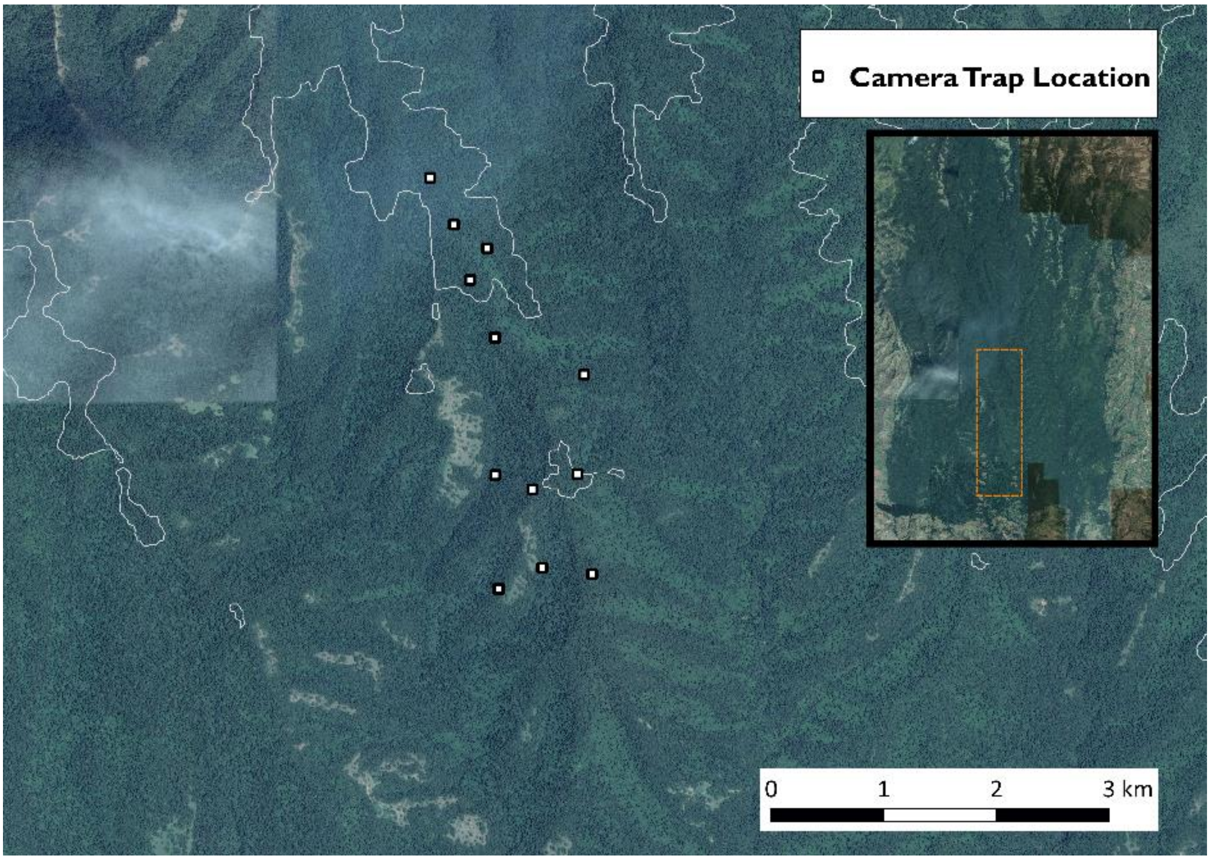

*Figure 2: Camera Trap locations in the grid survey conducted in Maasai Mau. Contour lines follow 300m elevation gradient. The inset shows the location within the Maasai Mau area.*

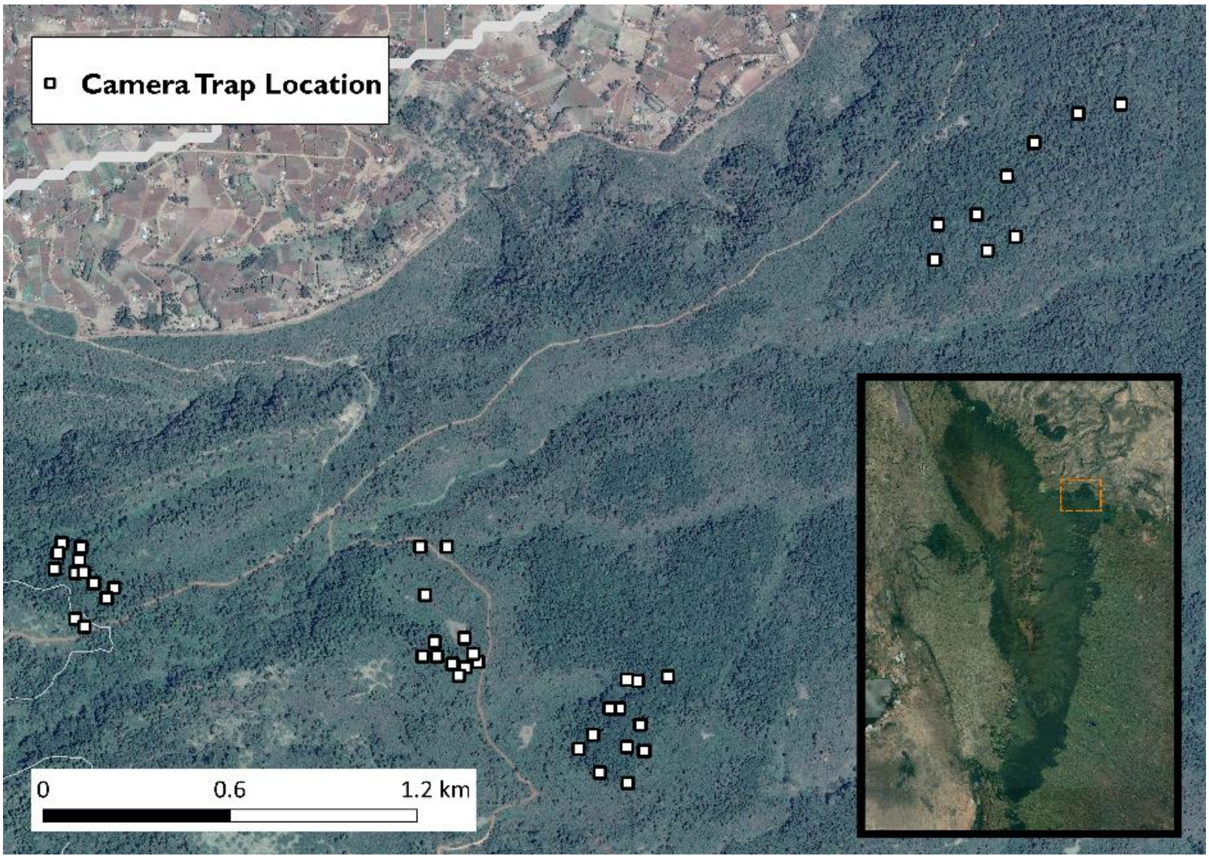

*Figure 3: Location of Deliberately Biased surveys conducted in the Salient zone of the Aberdares. The inset shows the location within the Aberdare range.*

In all the described surveys, devices are installed facing active game trails and tied on robust trees (to limit wind interference) and at a height of at least 1.5 – 2 m, to avoid disruption from animals (Meek et al., 2014). All camera trap surveys used Bushnell Natureview HD or Bushnell Essential HD cameras. Cameras were programmed to take three photographs per capture and were active for the entire survey duration. Shutter speed was set to high and infrared flash to high. When placed, all cameras were tested with a “walk test”: a person would walk in front of the device crouching at a height comparable to that of adult bongos (1.1 – 1.2 m, Elkan and Smith, 2015). Pictures resulting from this test were then assessed with either a laptop or a digital camera to perfection placement and orientation (Meek et al., 2014).

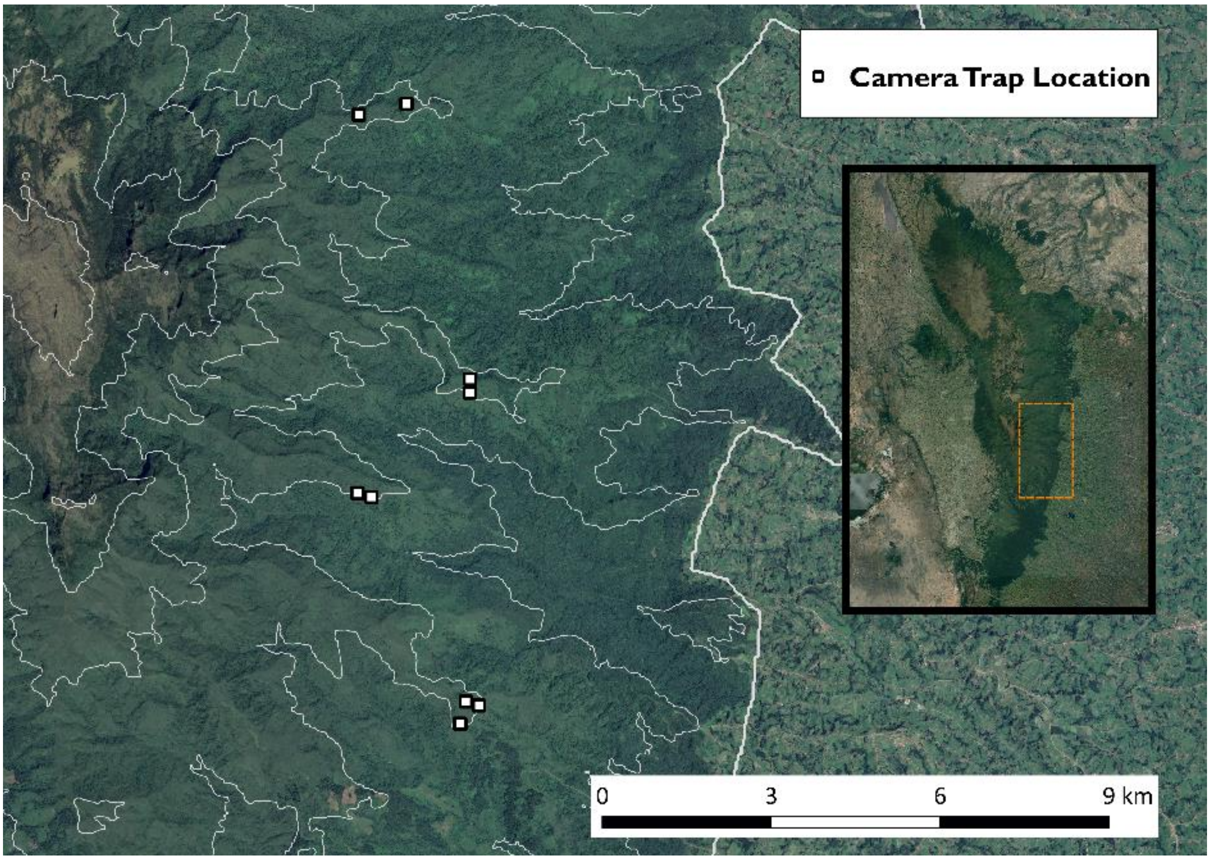

*Figure 4: Location of Non-Random surveys conducted in the Southern zone of the Aberdares. The inset shows the location within the Aberdare range.*

*Table 1: Details of surveys conducted between 2014 and 2018. Surveyed area is missing for deliberately biased (DB) surveys.*

| Survey (Year) | Area | CT N | Duration | Total Effort | Elevation Range | Area ($Km^2$) |
|---|---|---|---|---|---|---|
| Grid A (2015) | Salient ANP | 15 | 12 | 180 | 1970 - 2140 | 8.8 |
| Grid B (2015) | Salient ANP | 15 | 15 | 225 | 2390 - 2740 | 8.9 |
| Grid C (2015) | Salient ANP | 12 | 19 | 228 | 3150 - 3340 | 5.8 |
| Grid D (2016) | North ANP | 9 | 15 | 135 | 2280 - 3020 | 4 |
| Grid E (2016) | North ANP | 9 | 14 | 126 | 2700 - 3040 | 3.8 |
| Grid F (2016) | North ANP | 9 | 14 | 126 | 3220 - 3400 | 3.7 |
| Grid G (2018) | Maasai Mau | 12 | 13 | 156 | 2270 - 2460 | 2.7 |
| Grid H (2018) | Salient ANP | 12 | 25 | 300 | 2150 -2300 | 6.8 |
| South (2017) | South ANP | 9 | 33 | 297 | 2700 - 2770 | - |
| DB 1 (2014) | Salient ANP | 12 | 6 | 72 | ~ 2400 | - |
| DB 2 (2014) | Salient ANP | 12 | 6 | 72 | ~ 2400 | - |
| DB 3 (2014) | Salient ANP | 11 | 6 | 66 | ~ 2300 | - |
| DB 4 (2016) | Salient ANP | 9 | 6 | 54 | ~ 2200 | - |

### *Sign Surveys*

I conducted sign surveys following a recce approach (Walsh and White, 1999; Hausser et al., 2017) walking along game trails. Signs typically used for antelope detection are dung piles and tracks (Estes et al., 2008; Bowkett et al., 2009). However, identification of antelopes from dung is difficult without the use of genetic tools (Bowkett et al., 2009). This is a particular issue for bongo, as dung is similar in appearance to that of the syntopic waterbuck (*Kobus ellypsiprymnus*), a similarly sized antelope, which is known to result in misidentification (Faria et al., 2011). Therefore, only bongo tracks were used to identify presence. These were identified using a quantitative method I developed relying on captive populations of bongo and waterbuck (Chapter 2). The method discerns tracks of bongo from those of waterbuck relying on their aspect ratio (maximum length / maximum width). Recces were conducted in the Aberdares in 2017 and 2018 (Figure 5) and Maasai Mau in 2018 (Figure 6). In addition to recces, I also opportunistically recorded signs encountered during camera trap placement in all surveys.

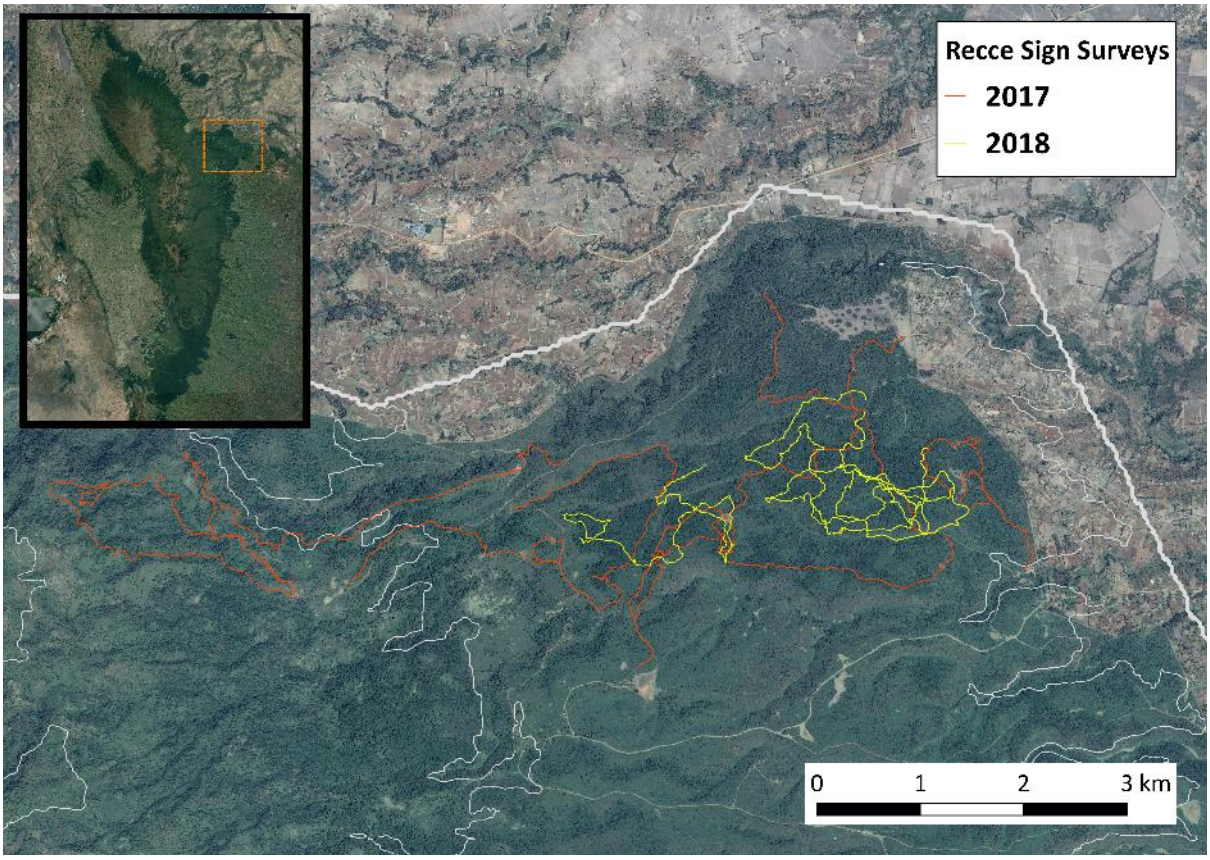

*Figure 5: Recce Sign surveys conducted in the Salient zone of the Aberdares. The inset shows the location within the Aberdare range.*

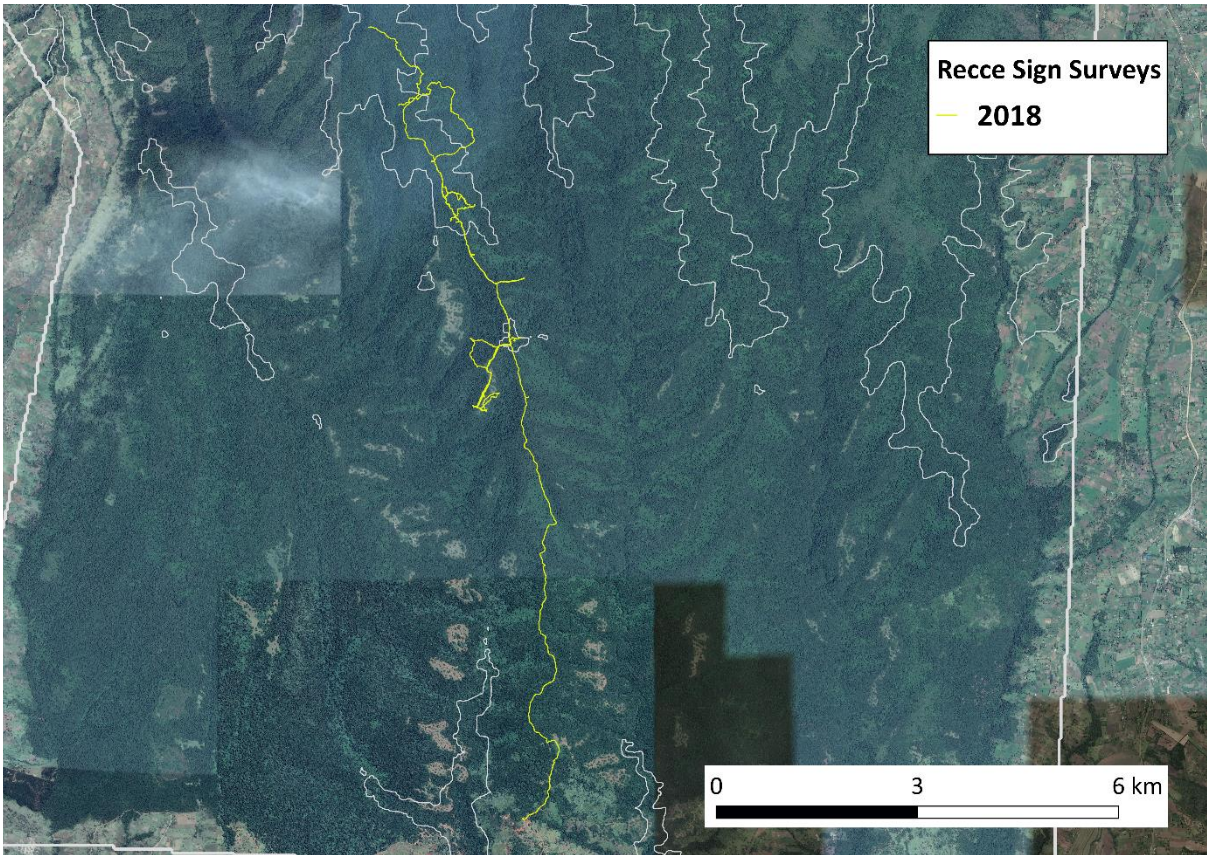

*Figure 6: Recce Sign surveys conducted in Maasai Mau.*

*Human Disturbance*

Signs of human activity were recorded opportunistically when conducting both camera trap and recce sign surveys. Typical signs of human activity in the area of interest consist of illegal selective logging (i.e. logging that focusses on trees of a certain species and of a certain size only) for red cedar (*Juniperus procera*) timber, and charcoal production (Rhino Ark, 2011; Massey et al., 2014). Additionally, poaching activity with snares, traps, and dogs happen in the areas covered by this study (Prettejohn, 2008). All camera locations and signs were georeferenced with a handheld GPS to within 3 – 5 m.

All fieldwork was conducted under research permit NACOSTI/P/17/16140/16909 and NACOSTI/P/18/16140/21823.

*Satellite Imagery and Remote Sensing*

In order to predict bongo habitat throughout the known range I retrieved four 30m resolution multi-spectral images from the Landsat 8 satellite (Roy et al., 2014, scenes ID: LC81680602017010LGN01, LC81680612017010LGN01, LC81690602017017LGN01, LC81690612017017LGN01, courtesy of the U.S. Geological Survey), acquired on 11 March 2017. All images were partially or totally cloud-free above the areas of interest (Mau, Eburu, Mt. Kenya and Aberdares). I merged the four images and clipped them to the areas of interest. I converted the resulting image to Top of Atmosphere Reflectance (TOA) using the package RStoolbox (Leutner et al., 2019). TOA is a transformation system that allows excluding atmospheric influence on the value of a pixel in a satellite image, an important step when working with multiple images (Wegmann et al., 2016).

The multiple wavelength of light in a multi-spectral image (called bands) can be combined to reflect specific characteristics on the ground, the so-called spectral indices (Wegmann et al., 2016). A particular set of spectral indices relate to vegetation in the area of interest, so called vegetation indices (Silleos et al., 2006; Xue and Su, 2017). Vegetation indices recovered from Landsat images rely on the bands corresponding to the wavelengths of green (band 3), red (band 4), near infrared (band 5), and middle infrared (band 7). We know of the importance of vegetation for bongo presence thanks to previous research (Estes et al., 2010, 2011; Sandri, 2014). The index mostly used in SDMs is the normalized difference vegetation index (NDVI). However, NDVI saturates in case of dense and multi-layered canopy (Haboudane et al., 2014). Because of the fine spatial scale (30m$^2$), I was interested in indices that could be sensitive to different vegetation structures. Therefore, I selected an arbitrary set of indices, retrieved from literature, combining the various bands associated with vegetation (3, 4, 5, and 7; Table 2). Furthermore, I implemented Tasseled Cap Transformation (TCT, Baig et al., 2014) in order to retrieve additional information from the multi-spectral images. TCT extracts information from a multispectral image and translates this into three variables each including a particular information: greenness (TCG, refers to vegetation presence), wetness (TCW, water content) and brightness (TCB, soil exposure). I used functions in the RStoolbox (Horning, and Schwalb-Willmann, 2019) to obtain vegetation indices and conduct TCT on the Landsat images.

*Table 2: Set of Spectral Indices and Topographic measures used. Variables in Italics show low collinearity and were used in model selection, the bands from multi-spectral Landsat image used in the calculation of the index, and the reference of the index first use or development. Indices in Bold Italic script are the ones employed in the SDM development.*

| RS Measure | Relates to | Satellite Imagery | References |
|---|---|---|---|
| ***Reflectance Ratio (RR)*** | distinguishes complex forest structure | Landsat8, bands 7 & 4 | Tonolli et al. 2011 |
| ***Normalised Difference Water Index (NDWI)*** | water content of vegetation | Landsat8, bands 5 & 7 | Gao 1996 |
| ***Modified Simple Ratio (MSR)*** | vegetation cover, sensitive to forest | Landsat8, bands 5 & 4 | Chen and Street 1996 |
| ***Normalised Canopy Index (NCI)*** | vegetation cover | Landsat8, bands 7 & 3 | Vescovo and Gianelle 2008 |
| Red Green Index (RGI) | sensitive to multiple foliar pigments | Landsat8, bands 3 & 4 | Coops et al. 2006 |
| Green Normalised Vegetation Index (GNDVI) | Vegetation cover | Landsat8, bands 5 & 3 | Gitelson, Kaufman, and Merzylyak 1996 |
| Corrected Normalised Difference Vegetation Index (NDVIC) | Vegetation cover | Landsat8, bands 5 & 4 & 7 | Nemani et al. 1993 |
| ***TCB*** | brightness, relates to soil cover | Landsat8, bands 2: 7 | Cohen et al. 1995 |
| TCG | greenness, relates to vegetation cover | Landsat8, bands 2: 7 | Cohen et al. 1995 |
| TCW | wetness, relates to water content in vegetation | Landsat8, bands 2: 7 | Cohen et al. 1995 |
| ***Elevation*** | Digital Elevation Model (DEM) 30m | SRTM 30m DEM | SRTM |
| ***Ruggedness*** | Slope of each pixel | SRTM 30m DEM | SRTM; Estes et al. 2011 |

Previous research has shown the importance of topography for bongo presence (Estes et al., 2011). Hence, I relied on a 30m resolution digital elevation model (DEM) retrieved from the SRTM. The image (encompassing the four areas of interest) was clipped to the bongo known areas. In this study, I relied on elevation and terrain ruggedness, expressed by calculating the slope of each pixel (the angle between a pixel and the 8 surrounding it). Following Estes (2011) slope values were averaged through a moving window of 15 pixels using RStoolbox, thus each 30m pixel value results from the average of the neighbouring 15 pixel, resulting in the average ruggedness of a 450 square meters area. The reason is that Slope is likely to influence bongo presence at a larger scale than the 30m resolution (Estes et al., 2011). All other variables were kept at the original 30m resolution. In order to assess disturbance in GIS, I used the locations of human activity to create a distance raster, which consists of an image where the value of each 30m pixel corresponds to the Euclidean distance from the closest disturbance location.

### *Ground Vegetation*

In order to ground truth findings from RS analysis, at each camera trap location in the Aberdares I sampled vegetation characteristics. Vegetation plots of 22.6m (0.04 Ha) diameter were measured, an area comparable to that of a 30m pixel (Estes et al., 2010). The vegetation features recorded were multiple. Basal Area: derived from diameter at breast height (DBH) of trees and shrubs with DBH > 7.5cm. Stem Density: calculated as the number of stems with DBH > 7.5cm in the plot, then expressed as density per hectare. Mid-Layer density: calculated through a 2m long pole with 40 visible signs spaced at 5cm apart held parallel to the ground at 1m height and assessed at the edge of the plot in all four cardinal directions; mid-layer density is then measured as the inverse of the proportion of visible signs out of the total 40. Herbs ground cover: measured at every meter along the diameters of the plot in the four cardinal directions, each meter I assessed ground cover to record if herbs were the major cover. The final measure consist of the proportion of ground covered by herbs and forbs. Differences in vegetation structure between points with bongo captures and absence points were evaluated using both Wilcoxon tests and logistic regression.

I conducted all spatial analysis in QGIS (QGIS Development Team, 2019) while I used R (R core team, 2019), for other spatial analysis, statistical analysis and data manipulation.

### *Model Building*

To identify important bongo habitat variables, I implemented Random Forest (Breiman, 2001), which has proved to be a powerful method in the development of SDMs (e.g., Elith and Graham, 2009; Evans et al., 2011; Guillera-Arroita et al., 2015). One limitation of machine-learning approaches like Random Forest is their low transferability in space (Randin et al., 2006). However, considering the aim here is to analyse and predict habitat suitability in a restricted area at the landscape level, transferability was not considered an issue. Moreover, the use of a complex model, which is better at identifying non-obvious relationships among variables, can produce better predictions, particularly when working on rare and endangered species (McMahon et al., 2014). Although Random Forest is robust to collinearity amongst explanatory variables, inclusion of collinear variables can decrease the model performance and complicate interpretation (Evans et al., 2011). Therefore, the set of variables retrieved from remote sensing tools (vegetation indices and topography measures) were assessed for their Variance Inflation Factor (VIF) (Dormann et al., 2012). The predictors were screened using the vifstep function in the package {usdm} (Naimi et al., 2014). I used a threshold of 10 for VIF to exclude highly collinear variables (Naimi et al., 2014; Cavada et al., 2017). Values of the uncorrelated variables were extracted for each camera trap and sign location in the dataset using the {raster} package (Hijmans 2017).

I used sampled vegetation measures to ground-truth measures from remote sensing (i.e. the vegetation indices). Values for each index with no collinearity issue (VIF < 10) were tested for correlation to the four ground vegetation variables (Stem Density, Basal Area, Mid Layer Density, and Ground Cover). As these measures were found to be not normally distributed in the dataset (Anderson Darling test $p < 0.05$) I relied on Kendall's tau to assess correlation (Kloke and McKean, 2015). Only indexes that correlate significantly with at least one of the ground measures were included in the model building process. This would facilitate interpretation of the predictions by relating the remotely sensed indices to the vegetation characteristics relevant for bongo habitat.

To conduct cross-validation of the developed SDM, data were divided into a train dataset (70% of locations) and a test dataset (remaining 30%). The train dataset is used for developing the model while the test for model validation. Data partition maintained the same representation of the two areas (Aberdares and Maasai Mau) as in the original dataset. Random Forest can handle many predictors, however a model with fewer variables in easier to interpret (Murphy et al., 2010). Thus, to find the best model for predicting bongo habitat I followed the parsimonious model selection approach described by Murphy (2010). Multiple models with different sets of variables are iteratively run and assessed for both their out-of-bag (OOB) error (i.e. the error of assigning the right class to the withheld data during bootstrapping) and within-class error in a confusion matrix (Murphy et al., 2010; Evans et al., 2011). The best model is the one that minimizes these errors while including fewer variables. The best set of variables found was implemented in a fit model that was then evaluated for its predictive ability against the test data.

The typical measure of predictive ability is the area under the curve (AUC) of a Receiver – Operator Characteristics (ROC) (Fielding and Bell, 1997). However, the use of AUC as a reliable measure of predictive ability has been criticised when used in rare species models

(Manel et al., 2001). Therefore, following Murphy (2010) and Evans (2011) I assessed model significance against a random model, and model performance through cross-validation against withheld data, each with 1000 iterations (Murphy et al. 2010). Moreover, following Manel (2001) I also use Cohen's kappa (Cohen, 1960) for model validation. Cohen's kappa (hereafter kappa) measures the proportion of all possible cases of presence or absence that are predicted correctly by a model after accounting for chance. The best model would then be used to predict suitable habitat throughout the bongo known range. The scale of the resulting SDM was maintained at 30m resolution. However, pixels were resampled using a moving window covering 15 pixels, as it is likely that the habitat in surrounding pixel influence habitat suitability of a single 30m pixel. I conducted random forest model selection, validation, and evaluation using the R package {rfUtilities} (Murphy et al., 2010).

To assess whether disturbance influences bongo presence I employ a generalised linear model with binomial distribution (i.e. logistic regression). The simple nature of this model allows testing against a null-hypothesis and results are easier to interpret than machine learning outputs (Elith and Graham, 2009). All the variables screened for VIF were included (without interactions) in an initial model that was then simplified using the stepAIC function in the package {MASS} (Venables and Ripley, 2002). The model with the lowest value for the Akaike Information Criterion (AIC) was considered the best fitting and its parameters were the best for explaining bongo presence. The most informative model would be tested against a second model that includes disturbance to assess the influence of this variable. Using an information theoretic approach I measured the AICc (i.e. second order AIC, suitable for small datasets, Anderson and Burnham, 2002) of the disturbance model and compared it to that of the model including only vegetation features (hereafter vegetation model). The difference in AICc would indicate whether the inclusion of disturbance made the vegetation model more or less informative. Furthermore, I tested the predictive ability of each GLM model (with AUC) to assess whether the inclusion of disturbance enhances predictive ability.

## Results

### *Camera Trapping*

Extensive camera trapping resulted in 17 bongo capture events in 13 camera trap locations. Of these, eight cameras were part of the “deliberately biased” surveys, while the remaining were part of the grids. All but one of the locations are in the Aberdares, Figure 7 shows the locations of these camera traps in Aberdares. The mean time a camera was operational until its first bongo capture was 8.2 days (*SE* ± 2.2, N =13). Therefore, in case of the short surveys, which lasted only 6 days, cameras that failed to detect a bongo were not counted as absence locations and were excluded from the analysis. The remaining surveys lasted at least 12 days, thus they were kept for the SDM development. Moreover, two cameras were lost, likely stolen, in two surveys (specifically Grid A and B, see Table 1), and their location was therefore excluded from the analysis. An additional point in Grid B was excluded due to the finding of bongo tracks in its vicinity (< 30m) in a recce conducted in 2017.

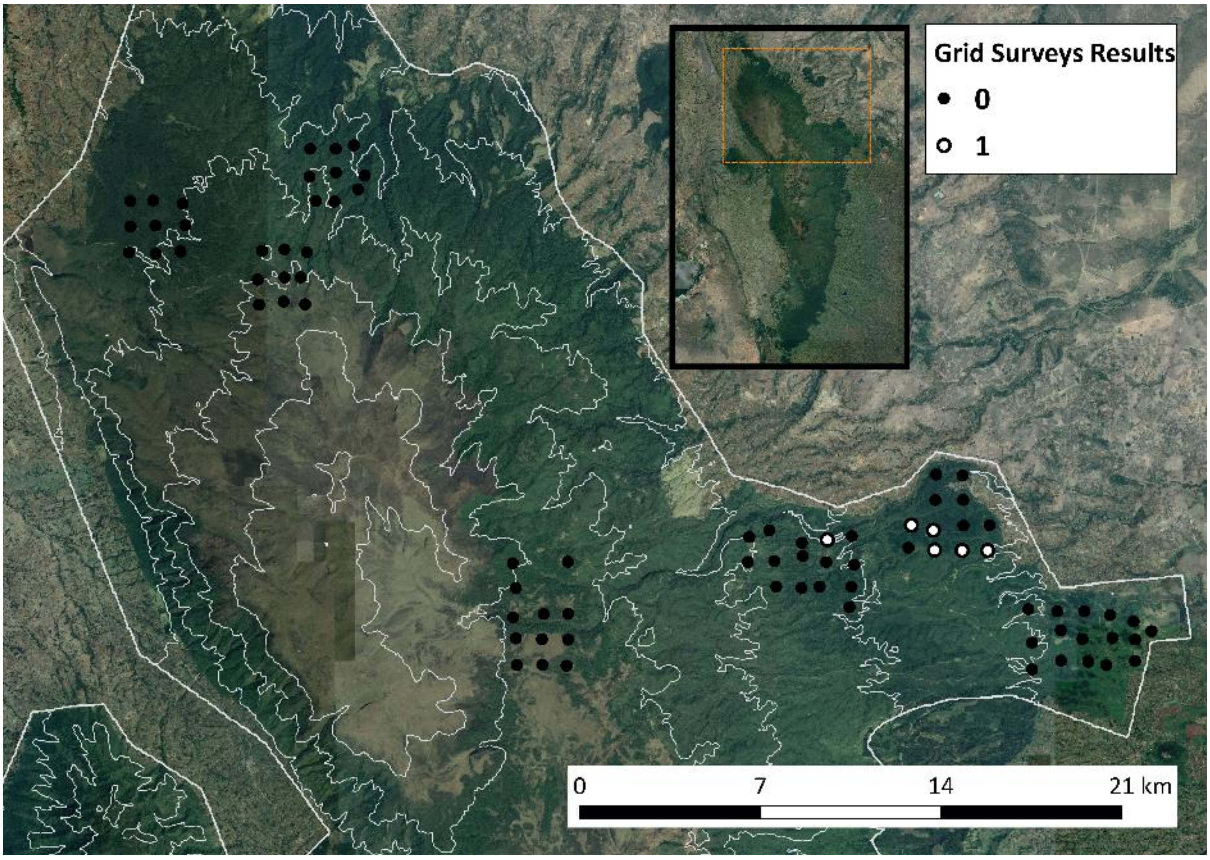

*Figure 7: Results of the grid surveys in the Aberdares. The inset shows the location within the Aberdare range.*

*Sign Surveys*

During the recce sign surveys, I recorded 104 tracks in Aberdares and Maasai Mau. In case of multiple tracks falling within a single pixel, checked visually in QGIS, only one was kept for further analysis. This resulted in 43 tracks in Maasai Mau and 35 in the Aberdares that I kept for SDM development. Figure 8 and 9 show track locations in the Aberdares and Maasai Mau respectively. The total of presence locations used in the SDM development is 91. The remaining cameras (n = 89) constitute the absence points used in the analysis. My data are balanced with close to 1:1 representation of presence and absence locations. This helps avoiding imprecise class predictions, a common issue in SDMs with unbalanced data (Evans et al., 2011).

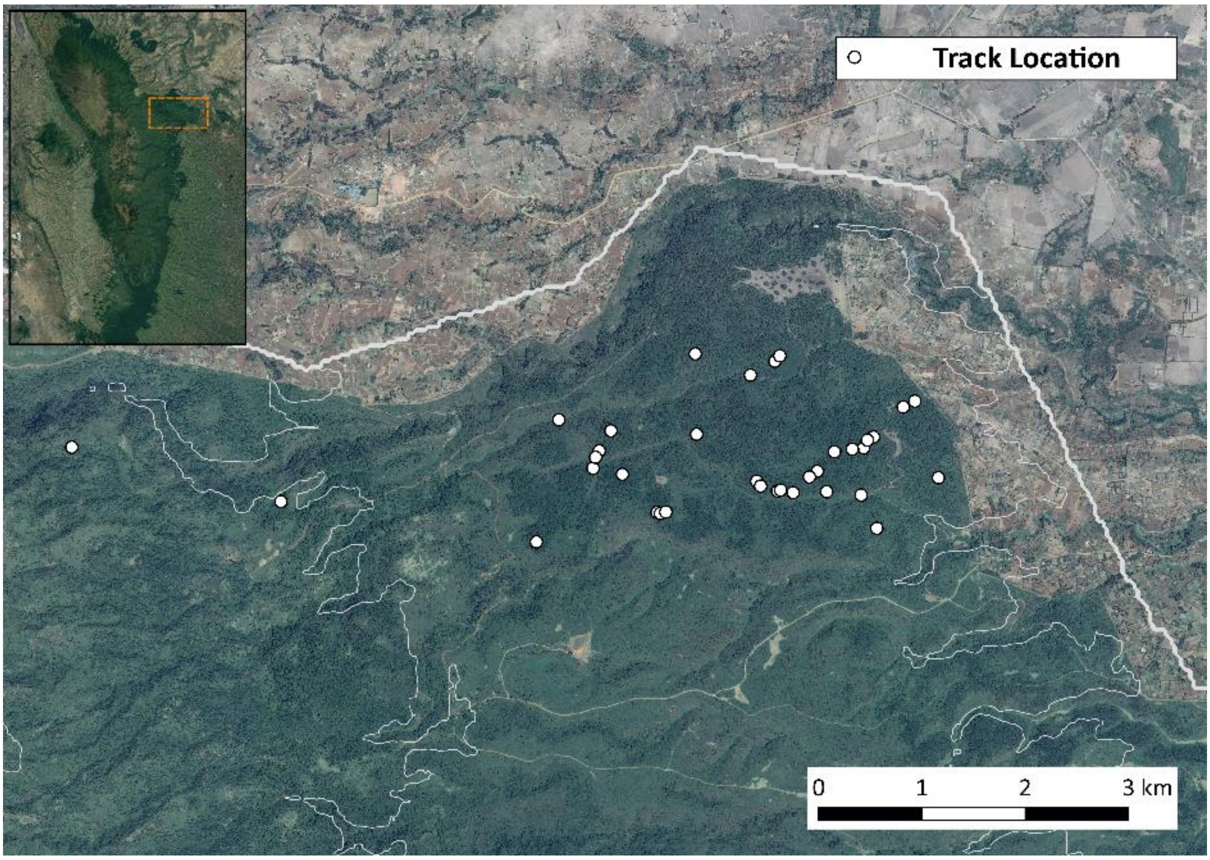

*Figure 8: Tracks recorded in the Aberdares. The inset shows the location within the Aberdare range.*

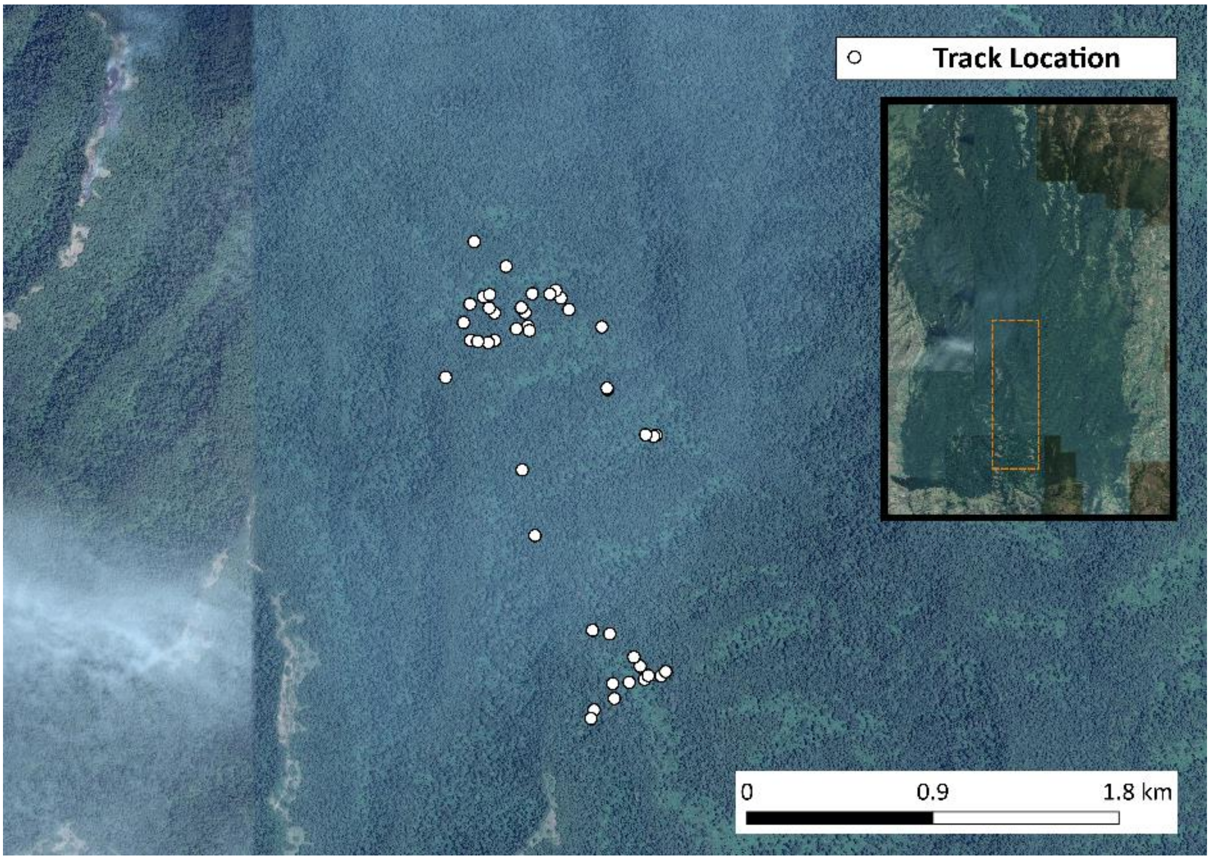

*Figure 9: Tracks recorded in Maasai Mau. The inset shows the location within the Maasai Mau area.*

### *Human Disturbance*

Signs of disturbance were found in both the Aberdares and Maasai Mau, with 34 points recorded. The majority of these (n= 28) consist of selective logging sites. Other signs found consist of human footprints accompanied by dogs (n = 2), and one recently build hut used by loggers, these signs were qualified by experienced rangers. A single rope-snare was found in the southern Aberdares. When conducting my survey and recces in Maasai Mau, I found that a disused road is a main access point to the forest, and multiple logging sites were found on the roadsides. Therefore, I counted the entire road (tracked via GPS) as a single disturbance location. Due to the majority of disturbance locations being due to selective logging, the analysis of the influence of disturbance should be seen as the influence of selective logging on bongo presence, rather than human disturbance at large.

### *Model Building*

Of the 13 initial variables, described in Table 2, seven showed no issue of high collinearity: Elevation, Ruggedness, and the vegetation indices TCB, MSR, RR, NDWI, and NCI. All these vegetation indices are significantly correlated with vegetation measured on the ground (Table 3). Parsimonious model selection (Murphy, 2010) resulted in a model containing five variables, including elevation, MSR, TCB, ruggedness, and RR, which varied greatly in relative importance and partial dependency for bongo presence (Figure 10). The model has an OOB error rate of 13.4%, with a correct classification rate of 82.8% for absences and 90.3% for presences. Model predictive performance was good when tested on the test dataset with an AUC of 0.92. The model was significantly different to a random model ($p < 0.05$) and the cross-validation results show a kappa for the model of 0.7, which is considered a substantial model performance (Landis and Koch, 1977; Manel et al. 2001). In order to address the influence of different efforts among surveys, I repeated the model building considering any late capture (beyond 15 days) as absences. The model developed using this other dataset retained the same predictors as the full dataset.

*Table 3: Correlation among ground vegetation measures and spectral indices. As ground measures are not normally distributed, Kendall's tau was implemented. The asterisks indicate significant correlation at p < 0.05. Indices in Bold Italic script are the ones employed in the SDM development.*

| **Spectral Index** | **Stem Density** | **Basal Area** | **Mid Layer Density** | **Herbs Cover** |
|---|---|---|---|---|
| ***RR*** | - 0.06 | -0.16 * | 0.18 * | - 0.2 * |
| ***NDWI*** | -0.17 * | 0.07 | -0.05 | 0.08 |
| ***MSR*** | 0.26 * | -0.03 | -0.02 | -0.04 |
| ***NCI*** | -0.32 * | -0.28 * | 0.22 * | -0.2 * |
| ***TCB*** | -0.3 * | -0.33 * | 0.16 * | -0.18 * |
| NDVIC | 0.45 * | 0.13 * | -0.15 * | 0.02 |
| GNDVI | 0.17 * | -0.07 | 0.05 | -0.08 |
| TCG | 0.04 | -0.15 * | 0.09 | -0.1 |
| TCW | 0.39 * | 0.12 | -0.17 * | 0.1 |
| RGI | 0.39 * | 0.13 * | -0.15 * | 0.05 |

The model suggests three vegetation indices, which correlate to ground vegetation measures, are important in predicting bongo presence (MSR, TCB and RR), as well as elevation and ruggedness, which is consistent with previous results (Estes et al. 2010, 2011; see Figure 11). The SDM confirms the importance of vegetation for bongo presence: MSR is a good predictor of structured vegetation (Haboudane et al. 2004), and here correlates with stem density, while TCB relates to open ground (Baig et al., 2014) and here correlates negatively with forest indicative measures (i.e. stem density and basal area), and positively with mid layer density (i.e. shrubs). Moreover, the inclusion of RR, which refers to forest structure (Tonolli et al., 2011), and here is related to all measures except for stem density, further helps in refining the model prediction. Vegetation appears to be linearly related to bongo presence by the partial dependence of both MSR and RR indices (Figure7). While open ground is clearly avoided, as higher TCB values are related to absence of bongo (Figure 7). Moreover, topography is also relevant for bongo presence, as elevation was found to be the most important predictor, and, as shown in Figure 7, bongo occupy a specific altitudinal range (2000 – 2600 m). The importance of topography is also shown by the relevance of terrain ruggedness (Figure 7).

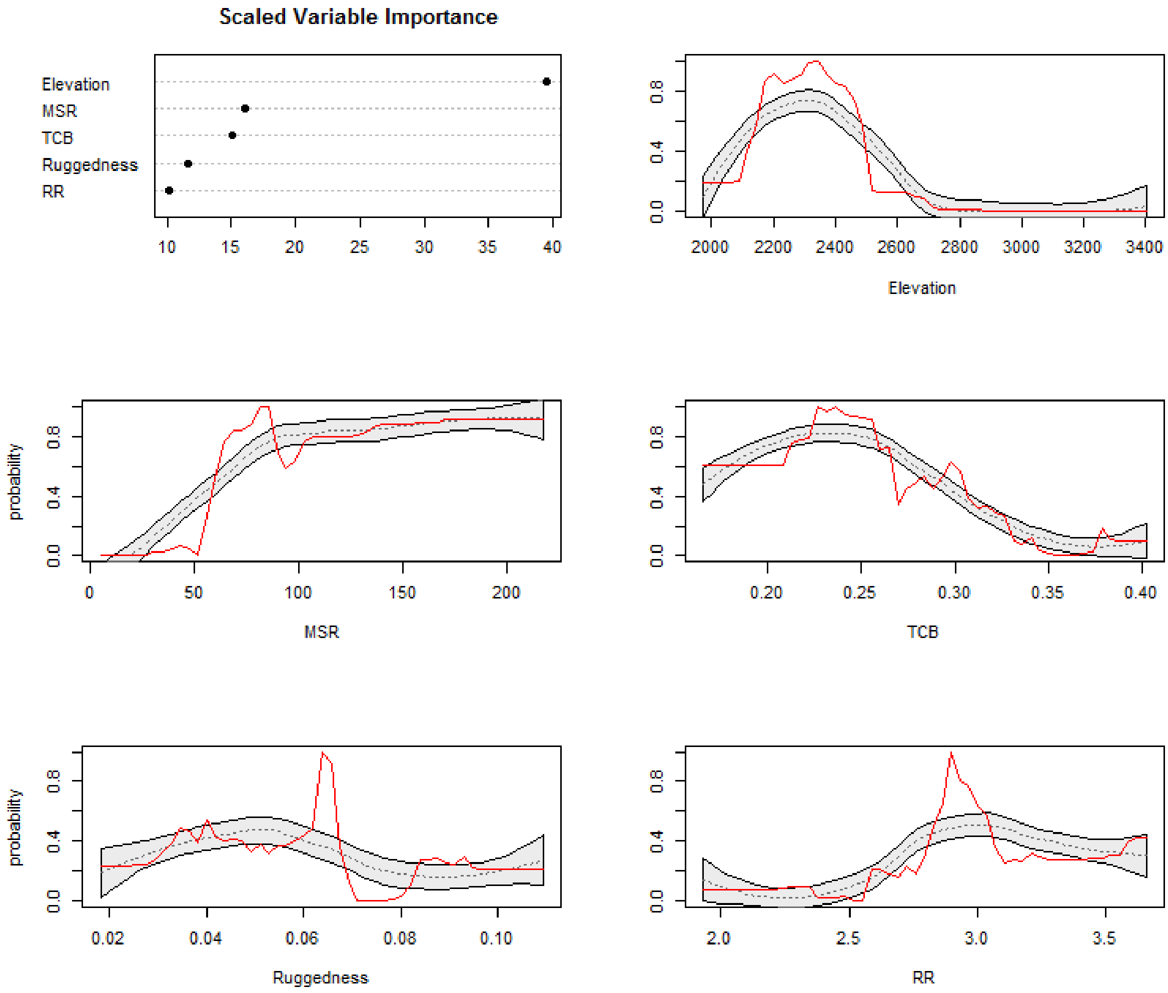

*Figure 10: Partial dependence plots for each of the variables included in the final RF model, and the scaled importance for each of the variables. Both raw (in red) and smooth line with confidence intervals are shown.*

I used this model to predict bongo habitat throughout the four areas of interest (Maasai Mau, Aberdares, Mt Kenya and Eburu). The maps were computed using a moving window approach covering 15 pixels, this would allow the identification of suitable areas in relation to the surrounding ones. Results for each area are shown in Figure 12 and 13.

In assessing the influence of disturbance on bongo presence, the best logistic regression found through stepwise variable exclusion and AICc evaluation included six of the variables contained in the initial full model. Results, shown in Table 4, show a difference in the AICc of 0.15. Therefore, the inclusion of disturbance fails to provide any additional information to the vegetation model. Moreover, the analysis of the predictive ability of the models showed it to be equal (AUC = 0.84). This indicates that distance from locations with disturbance, in this case mainly selective logging, does not influence bongo habitat selection.

*Table 4: Model Selection Table showing difference between the best GLM (found through a stepwise selection in R) and the same model with the addition of disturbance (measured as the distance from a location where human disturbance was detected) as a covariate. Results show that the inclusion of disturbance fails to provide the model with additional information.*

| **Model ID** | **N Parameters** | **AICc** | **Δ AICc** | **AICc Weight** | **Pseudo-$R^2$** |
|---|---|---|---|---|---|
| Best GLM plus Disturbance | 8 | 162.68 | 0 | 0.52 | 0.44 |
| Best GLM | 7 | 162.81 | 0.14 | 0.48 | 0.43 |

As per habitat selection on the ground, when assessed through both Wilcoxon test and logistic regression, results show that Stem Density is the only vegetation feature that differs significantly between presence and absence plots. Wilcoxon rank sum test shows strong significance ($p < 0.001$), as shown in Figure 11. This is also confirmed by the logistic regression analysis. The model built with Stem Density as predictor for bongo presence shows the lowest AICc when compared with models built with the remaining vegetation features, even when including covariates both with and without interactions. The results from my partial on the ground analysis confirm results from RS analysis, and previous studies (Estes et al., 2008).

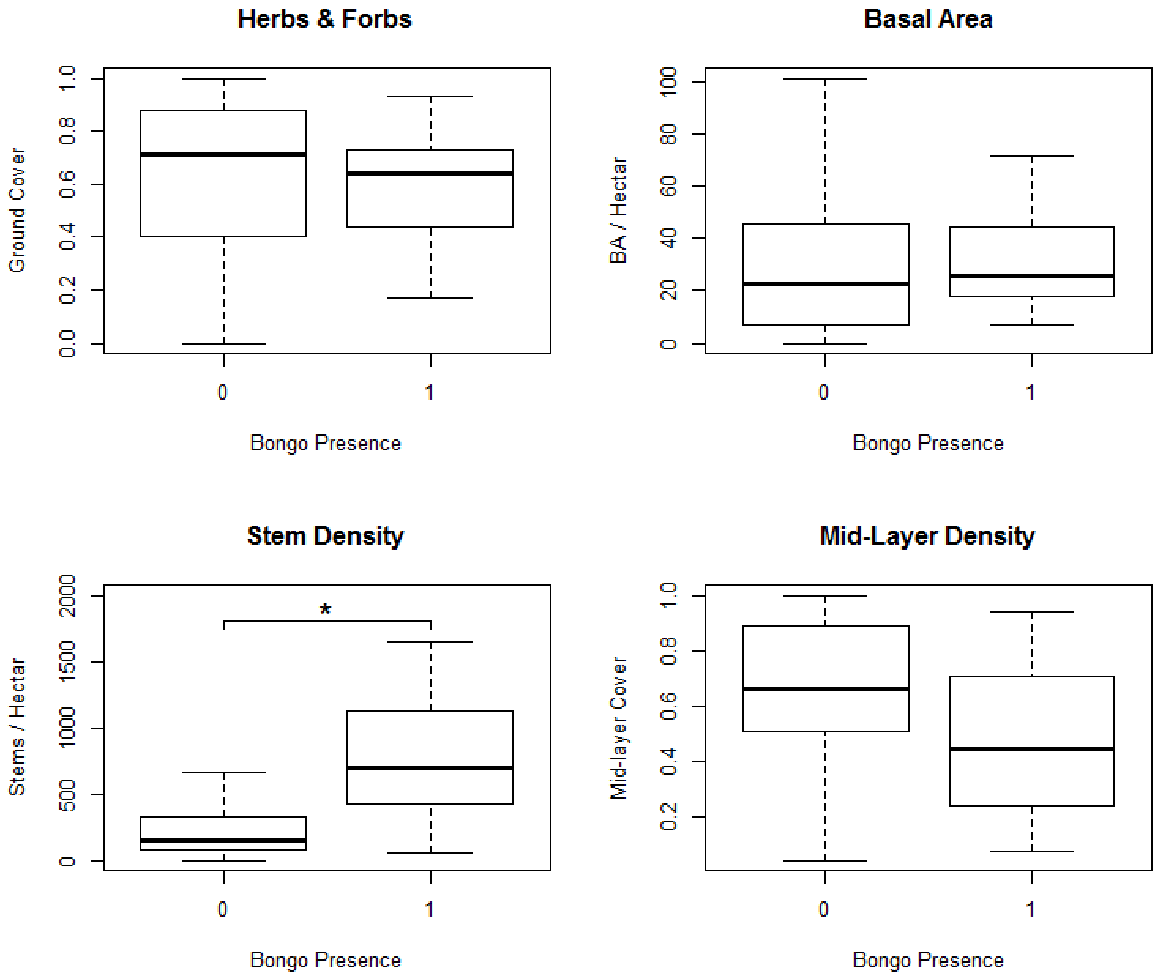

*Figure 11: Boxplots showing the difference between Presence and Absence plots in Vegetation Features recorded on the ground. Asterisks show differences found to be significant with a Wilcoxon test ($p < 0.001$).*

## Discussion

Here, I developed a SDM for the critically endangered mountain bongo. The model predictive ability is good with both a high kappa (0.7) and high AUC (0.92) (Manel et al., 2001). The SDM here presented can become a tool in the long-term conservation of this antelope, as it can direct current conservation efforts towards areas where bongos are likely to persist. Moreover, the predictions of suitable areas throughout the known bongo range can inform future effort aimed at supporting the recovery of bongo in the wild, like reintroduction of individuals from captive populations (Veasey 2010; Reillo 2002). These predictions are here based on locations where bongo are known to be breeding and populations are growing (Chapter 3). Hence, the predicted habitat is likely indicative of areas that would not only allow populations to persist, but also to thrive (Osborne and Seddon, 2012). The main limitation of the approach here followed is the strong localisation of presence points, as visible in Figure 7 – 9. However, considering the critical situation of this antelope, and considering that I only wanted to rely on growing populations for my prediction, an approach where samples would be located by any bongo sign throughout the range would have hampered the ability of the model to predict optimal habitat for bongo.

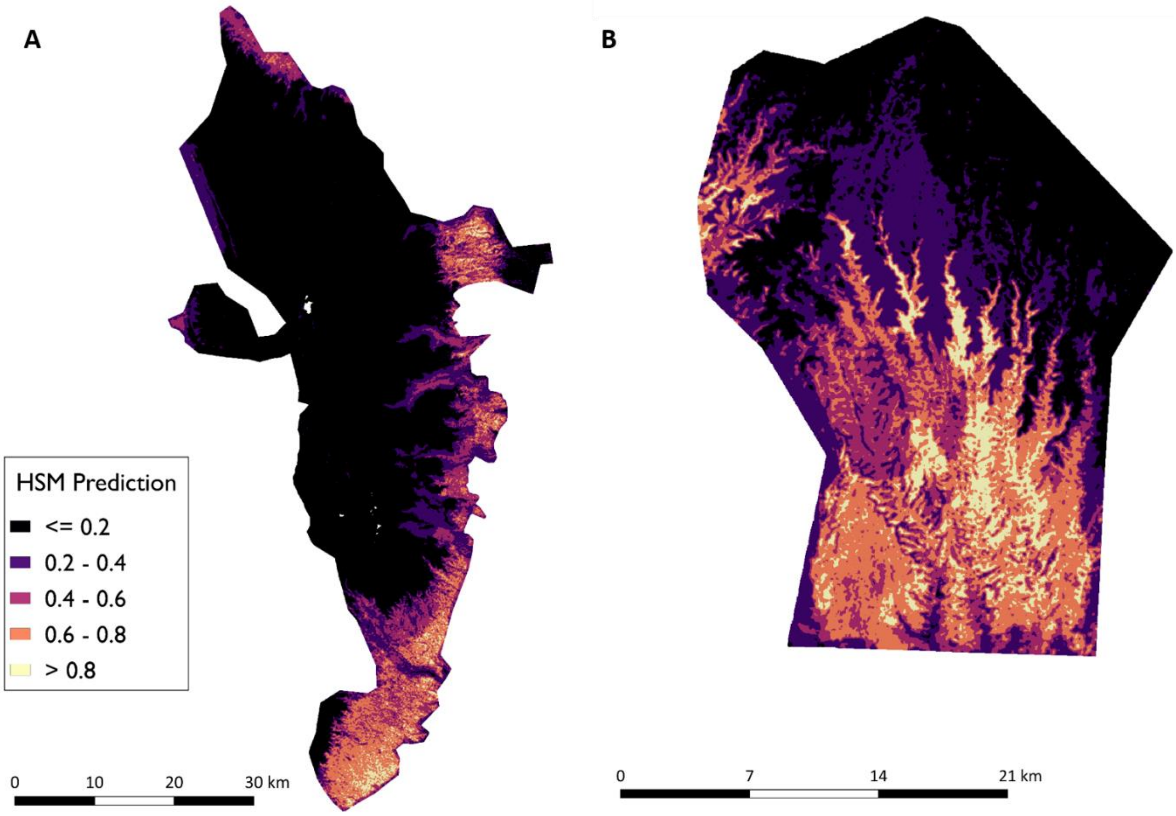

*Figure 12: SDM prediction in Aberdares (A) and Maasai Mau (B).*

While all the isolate mountains where bongo are currently found appear to have areas suitable for bongo populations, Eburu appears to have the least (Figure 13) whereas the Aberdares and Mt Kenya both have localised patches of suitable areas. In the Aberdares the area currently known to be occupied by bongo, the Salient (an eastern spur with an elevation span of 2000 – 3000 m), retains areas with suitable habitat (Figure 12). Moreover, the southern part of the Aberdare range, known as the Kikuyu escarpment, appears to host vast areas of bongo suitable habitat. This area is rarely mentioned as a location where bongo were present or as a possible reintroduction site, hence the need for further investigation and surveys in this part of the Aberdares. In Mt Kenya, the south and south-west slopes contain the areas with higher suitability (Figure 13). Of all the areas this study concerns, Maasai Mau appears to have the highest extent of suitable habitat (Figure 12). The importance of Maasai Mau for bongo conservation has already been raised when bongo demographics have been analysed (Chapter 3). Results of this study reiterate the relevance of this forest, which currently lacks formal protection, not only as a stronghold for bongo, but also as an area of possible expansion for the resident population and location for reintroduction, therefore the area should become a priority in the conservation of this iconic antelope.

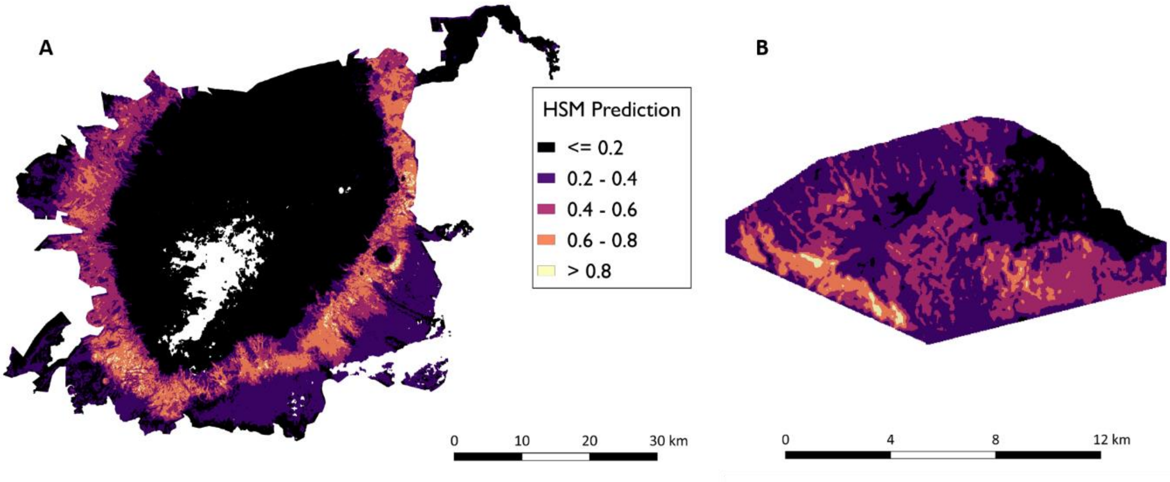

*Figure 13: SDM prediction in Mt. Kenya (A) and Eburu (B).*

An interesting result in this study is the little role disturbance plays in shaping bongo distribution. While literature mentions that the conspecific Lowland bongo (*Tragelaphus eurycerus eurycerus*) is attracted to logging areas due to the extensive regrowth typical of these areas (Estes 1992; Elkan and Smith 2013), I found no evidence of disturbed locations being relevant to bongo distribution. This result is interesting also in light of the difference seen in the data between Maasai Mau and the Aberdares, where in the former disturbance occurrences overlap with areas where bongo signs were detected, unlike in the latter. This shows that disturbance in the Aberdares happens in areas that host unsuitable bongo habitat, whereas in Maasai Mau disturbance occurrences were found in areas occupied by bongo. I focused on the impact of human disturbance found within the areas, which is mainly limited to illegal small-scale selective logging. My results are in accordance with studies where selective-logging resulted not to have a significant impact on species presence (Edwards and Laurance 2013). Nevertheless, my results reinforce the importance of vegetation and topography features for bongo presence, even when human disturbance, selective logging in particular, may be present. Hence, it appears that areas that seem disturbed may be good habitat for bongo, so long as the vegetation and topography are optimal. Moreover, distance from the border of the protected areas could have been implemented to better assess the influence of vicinity to local communities may have in bongo distribution, and consequently use this measure in the prediction on the entire range. However, the presence of a fence in the Aberdares (Rhino Ark, 2011) and not in Maasai Mau, and the unbalanced nature of the data between these two areas would have hampered a clear interpretation of the results.

The finding that vegetation structure and topography shape bongo habitat associations was expected after previous research results (Estes et al., 2008, 2010, 2011). The thorough work conducted by Estes clearly indicates that vegetation is the main driver of bongo habitat selection. Nevertheless, my findings differ to those of Estes in one key aspect: the influence of ruggedness. While Estes noted a preference in bongo for steep valleys, likely due to the higher security given by the terrain, I found the opposite, where bongo appear to avoid steep areas in favour of less rugged terrain. Estes found bongo in the north and south Aberdares, however both this study and the BSP have failed to detect them there in recent years. This may be due to those populations becoming extinct in the last decade or remaining at such densities that detection is difficult. However, both cases likely indicate that no breeding population currently occupy those areas. These areas are characterised by steep valleys, and thus may represent sub-optimal habitat once occupied by marginal populations. Whereas, my study predicts suitable habitat for bongo relying on presence locations retrieved in areas where breeding herds are present, it is therefore likely that steep valleys are not a core component of bongo habitat, but a refuge in marginal areas.

A common issue when developing SDM is the reluctance of managers and practitioners in incorporating these in their decisions (Anadón et al., 2010). Hence, the need for easy to interpret and yet powerful models. My SDM for bongo responds to both these needs, it is powerful in its predictive ability thanks to the use of random forest, and it is easy to interpret thanks to the use of a limited set of variables. Moreover, the model interpretability is heightened by having also conducted an analysis on ground vegetation measures, which were then related with the spectral indices on which the model relies. Practitioners and managers can therefore use the model to drive the investigation of suitable areas, and the search for yet not discovered remnant populations. Therefore, my SDM could become a valuable tool for the managers of the areas where this antelope still thrives, and be used in future conservation actions aimed at restoring it in its native range. Conservation actions have made a difference for ungulates in the wild (Hoffmann et al., 2015), and my results can be of help in allowing this to be the case for the critically endangered mountain bongo.

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# Chapter 5:
# Comparison of Wild and Captive Genetic Diversity in a Critically Endangered Antelope: Implications for Conservation Actions

### *Context in the Thesis*

*The previous chapters have focussed on providing missing information on the situation of bongo in the wild (Chapter 3) and updated current understanding of where suitable bongo habitat may be located (Chapter 4). Here, I focus on assessing the genetic diversity (measured at mitochondrial level) to try to mitigate concerns regarding the use of the captive population for reintroduction.*

### Abstract

The role of captive breeding for the conservation of endangered species is widely recognised, captive individuals can be used in actions that aim to restore or augment dwindling or extinct wild populations. However, such actions are often hampered by lack of knowledge of the genetic situation of both captive and destination populations, and the fear of outbreeding depression. The captive population of the Critically Endangered mountain bongo (*Tragelaphus eurycerus isaaci*) provides opportunities for the restoration and augmentation of the small, fragmented, and genetically depauperate remnant populations in the wild. Here, I assess the diversity found in the control region of mtDNA in the wild and captive populations in Kenya. Differences in mtDNA between wild and captive individuals may lead to mito-nuclear incompatibilities, which can cause outbreeding depression to occur. By assessing genetic diversity through the control region of mtDNA I integrate previous research to assess whether the reintroduction of captive bongo poses a risk of outbreeding depression. Results show that the majority of captive individuals, belonging to six of the 10 extant matrilines, represent just one of the only two haplotypes found in the wild. My findings and previous research indicate that using the captive mountain bongo population for reintroductions is unlikely to cause outbreeding depression.

## Introduction

The global loss of biodiversity is a major challenge facing our society, with extinction rates comparable to those of mass extinctions (Ceballos et al., 2015, 2017). Species-focussed conservation aims to prevent extinction of individual life forms by providing a framework for protecting populations of endangered species (Hoffmann et al., 2010). Captive breeding is an important tool in species conservation, with zoos being major actors in preventing extinctions (Conde et al., 2011; Fa et al., 2011). Zoos and captive breeding centres worldwide help the long-term conservation of species by maintaining captive populations, which can insure against the loss of their wild counterparts (Wayre, 1969). Moreover, captive bred individuals can also be used for reintroduction (Fiumera et al., 2000).

The role of captive populations is to preserve a reservoir of individuals and to maintain genetic variation that could be lost or under-represented in the wild (Fa et al., 2011; Leus et al., 2011). Low genetic variation is a major issue in the persistence of small populations, as lower diversity combined with inbreeding can facilitate the emergence of deleterious alleles which can directly affect population growth (Lande, 1988), and lead to extinction (Frankham, 2005). Captive breeding provides opportunities for augmenting or restoring genetic variation in small populations of conservation concern (Ralls et al., 2018; Bell et al., 2019). Captive bred individuals could therefore be valuable for the persistence of wild populations.

A common concern in relying on translocation of individuals from different populations for rescue actions, be they demographic or genetic, or even augmentation of population size, is the risk of outbreeding depression (Edmands, 2007; Bell et al., 2019). This can result when individuals from genetically distinct populations are mixed, and their progeny acquire a lower fitness (Lynch and O'Hely, 2001). This lower fitness may be due to differences in their genetic makeup, particularly in karyotype, or them being adapted to different environments (Frankham et al., 2011). Such concerns include the use of individuals from captivity, as these may be originally from a distinct population to the one where they would be reintroduced. Therefore, conservationists must research whether or not outbreeding depression is an issue. In order to limit this risk, Frankham et al. (2011) provide a framework of conditions under which outbreeding depression would be expected. While breeding between populations of the same taxon (i.e. species or subspecies) rarely leads to outbreeding depression (Whitlock et al., 2013), the fear of outbreeding leading to a lower fitness is limiting the implementation of reintroduction and assisted gene flow in endangered species (Ralls et al., 2018). Hence, providing managers with information on the genetic makeup of populations can provide reassurance and encourage them to take vital actions.

An underappreciated issue in evaluating the risk of outbreeding depression in reintroduction efforts is the impact females, specifically their mitochondrial genome, can have on the rescued population (Havird et al., 2016). Mitochondrial genomes undergo natural selection in creating optimal combination with the nuclear genome in developing so-called mitonuclear complexes that are relevant for core metabolic functions (Hill et al., 2019). Infusing novel or foreign mitochondrial DNA into a population through introduced females may cause mitonuclear incompatibilities, which can ultimately affect individual fitness (Gemmell et al., 2004). Therefore, even when outbreeding depression seems unlikely considering the time two populations have been separated or their unambiguous taxonomy (Frankham et al., 2011), analysing genetic diversity found at the mtDNA level can elucidate the risks of outbreeding depression. While such an issue can be avoided by just including males in conservation actions, in the case of critically endangered species there may be a need for individuals of both sexes to be reintroduced.

The mountain bongo (*Tragelaphus eurycerus isaaci*, hereafter bongo), is a large antelope endemic to the highland forests of central Kenya (Kingdon, 1982; Estes, 1992). Once widespread from Uganda to Kenya, the bongo suffered a severe decline throughout the 20th century due to a combination of habitat fragmentation, hunting, and disease outbreaks and its current distribution in the wild is limited to four isolated mountain areas: Aberdares, Mt. Kenya, Eburu, and Maasai Mau (Faria et al., 2011; Elkan and Smith, 2013). IUCN categorises this antelope as Critically Endangered due to the combination of a fragmented distribution and fewer than 50 individuals estimated to remain in the wild (IUCN SSC Antelope Specialist Group, 2016). Recent findings suggest that only two of these mountain areas host breeding populations: Aberdares and Maasai Mau (Chapter 3). The same study also found that fewer than 40 individuals may remain within these breeding populations, thus confirming the critical situation of this antelope.

In addition to its small size, the wild bongo population shows an extremely low genetic diversity as represented by mtDNA variation in the control region (Faria et al., 2011). Faria (2011) found only two haplotypes among the four areas, with only the Aberdares having both haplotypes represented; in comparison, 23 haplotypes were found in the populations of a sympatric antelope of least conservation concern, the waterbuck (*Kobus ellipsiprymnus*). Such a low genetic diversity, combined with the small size of the remnant populations, obviously imperils the persistence of bongo in the wild. Recent findings have shown that while the Aberdares remains the stronghold for this antelope, the Maasai Mau still has a breeding population and the presence of optimal habitat (Chapter 3 and 4). However, the genetics of this population has not been fully assessed and this could be of considerable importance for the long-term conservation of this antelope.

Nevertheless, the presence of a large captive population provides hope for the implementation for conservation actions. The bongo captive breeding programme currently holds more than 700 individuals in zoos and other facilities worldwide (Bosley, 2016). Such a large captive population provides an opportunity for undertaking conservation actions (East, 1999) where captive bred individuals could be used for genetic rescue and/or to augment wild population size through reintroduction (Price, 2016). Indeed, there has been one attempt with the bongo repatriation project of 2004 (Reillo, 2002; Veasey, 2010) where individuals from zoos and other facilities in the US were translocated to the Mount Kenya Wildlife Conservancy (www.animalorphanagekenya.org, hereafter MKWC), a captive breeding facility in Nanyuki, Kenya, as part of a planned future reintroduction. While the translocated individuals reinforced the captive population in Nanyuki, now consisting of more than 70 individuals, no reintroduction in the wild was attempted.

An analysis of the genetic diversity measured at the mtDNA control region in a sample of captive individuals in European zoos only recovered one of the two haplotypes described in the wild (O'Donoghue et al., 2017). Although the study claims that its sample is representative of the founders, it did not rely on female founder lineage (hereafter matriline) to assess founder representation and was only concerned with individuals from European collections.  Due to its maternal inheritance and absence of recombination, each matriline may represent a unique haplotype (Willoughby et al., 2015). Hence, when measuring genetic diversity from mtDNA in captivity, the only representation needed is that of female founders. A wider coverage of the living matrilines would allow a better understanding of the current genetic diversity held in bongo captive populations, particularly those held in MKWC, which are at the forefront of any conservation initiative.

Considering the limited period which separates the captive and wild bongo populations (~ 40 – 50 years) which is less than the 500 years suggested by Frankham (2011), and their pertaining to the same taxon (all captive individuals belong the *isaaci* subspecies as the founders were all sourced in Kenya), there is limited reason for outbreeding concern in implementing conservation actions. Nevertheless, the issue of possible incompatibilities in mtDNA in case of females being reintroduced is worth addressing. While recent findings show no significant differences between the Aberdares and MKWC populations when assessed through Single Nucleotide Polymorphisms (SNPs; Svengren et al., 2017), measuring diversity of mtDNA control region, of both the extant wild populations and the MKWC population will help in addressing any potential negative impacts of reintroductions. Although introducing only males would avoid mito-nuclear incompatibilities (Havird et al., 2016), given the critical situation of bongo wild populations, conservation actions would benefit from relying on individuals of both sexes.

Here, I analyse the genetic diversity of both wild and captive bongo population to inform the management of a critically endangered antelope regarding the risk of outbreeding depression. I will i) assess genetic diversity of the two remnant breeding populations in the Aberdares and Maasai Mau and that of the captive population held at MKWC and ii) integrate findings with results from previously sampled individuals from European facilities, in order to provide a better coverage of extant matrilines in comparison to the diversity found in Kenyan populations, both wild and captive. I discuss my findings in the context of bongo conservation genetics and the management of both captive and wild populations.

## Methods

The wild population was sampled to screen for mtDNA genetic variation. I collected samples from two populations, Maasai Mau and Aberdares. Dung samples were collected following a recce approach walking along game trails (e.g. Bowkett et al., 2009). The recces were conducted in areas known to be visited by the bongo herds in the two locations (Figure 1). Experienced trackers identified bongo dung. Dung was found in clusters, with identifiable individual piles, and samples were taken from each pile. These were collected using single-use sterile tweezers and stored in individual 50ml tubes filled with silica gel beads following Soto-Calderón (2009). Recces were conducted only in the Aberdares in 2017 while both areas were visited in 2018.

All fieldwork was conducted under research permit NACOSTI/P/17/16140/16909 and NACOSTI/P/18/16140/21823.

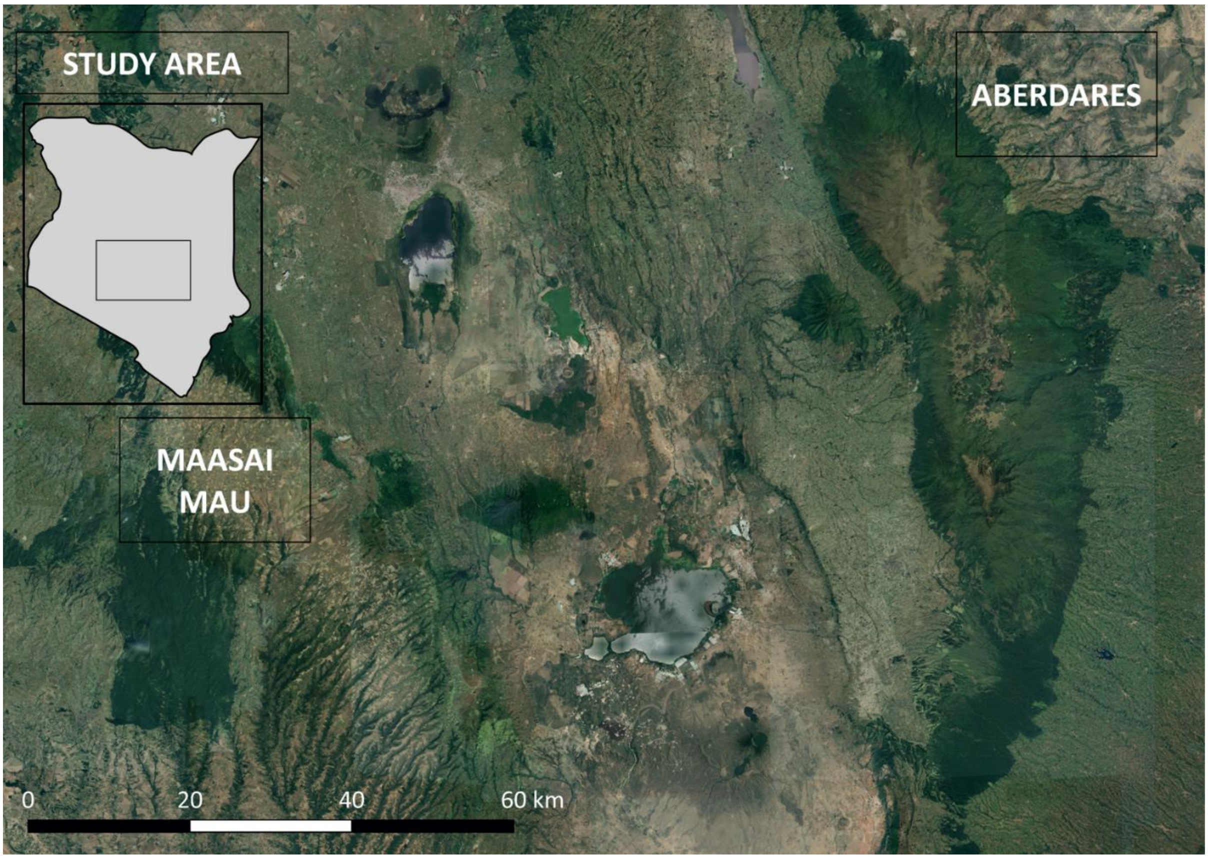

*Figure 1: The Study area, Maasai Mau and Aberdares in Central Kenya.*

For the captive bongo population I sampled individuals from different matrilines. Therefore, I identified living descendants of founding females by reconstructing the matrilines in the bongo pedigree relying on the historic version of the studbook (i.e. including all individuals that ever existed in the breeding programme; Bosley, 2016). Female founders were recognised using the GeneticsPed package (Gorjanc and Henderson,

2007) in R (R core team, 2019); here a founder is an individual with both parents recorded as being wild. Each female founder was assigned a lineage named after her studbook ID. Using custom R code all the descendants of each female founder were assigned to her lineage. In order to allow the full reconstruction of female lineages, I had to create two dummy founders to accommodate individuals with unknown dam, and hence unknown lineage. This was necessary for 274 individuals in the US portion of the studbook and for 108 individuals from MKWC.

Sampling of captive individuals was conducted at MKWC in Kenya in 2018. This population is of particular interest not only for its proximity to wild populations (it is likely that reintroductions will source individuals from here), but also because it has individuals which represent both local and US lineages (a result of the importation of bongos in the 2004 repatriation project (Veasey, 2010)). The sampling focused on obtaining maximum representation of founders, assessed through my identification of founder lineages. Opportunistic sampling of additional individuals was also conducted. Dung samples were collected relying on the MKWC keepers for individual identification. Dung was collected and stored as described for wild samples.

DNA extraction was performed in less than 3 weeks after collection in order to maximise yield (Soto-Calderón et al., 2009) using QIAamp DNA Stool mini kit (Qiagen, Valencia, CA, USA) with a modification in the protocol: a double elution was conducted using AE previously heated at 70 degrees. The two elutions were of 50 µl and preserved in separate tubes. Extracted DNA was then stored at -20 degrees awaiting export. Due to logistical restraints, I conducted a single extraction per dung sample.

*Table 1: ID and location of all individuals sequenced in the study. UF = Unknown Founder, where the original female founder of the individual was not traceable. MKWC = Mount Kenya Wildlife Conservancy, Nanyuki, Kenya. ID 185 is highlighted as it was not sequenced within this study (O'Donoghue et al., 2017). Individuals that could not be assigned to a particular lineage (Studbook ID 1902,1998,2190, 2272, 2299, 2783, 2859, 3015, 3141, 3152) are not shown.*

| **Studbook ID** | **Location** | **Matriline** | **Haplotype** |
|---|---|---|---|
| 1482 | Chester | 3 | B02 |
| 1616 | Chester | 3 | B02 |
| 1794 | Howletts | 3 | B02 |
| 1832 | Marwell | 3 | B02 |
| 1930 | Chester | 3 | B02 |
| 1972 | Antwerp | 3 | B02 |
| 2034 | Antwerp | 3 | B02 |
| 2056 | Givskud | 3 | B02 |
| 2079 | Chester | 3 | B02 |
| 2148 | Howletts | 3 | B02 |
| 2255 | Knowsley | 3 | B02 |
| 2259 | Woburn | 3 | B02 |
| 2266 | Antwerp | 3 | B02 |
| 2494 | Knowsley | 3 | B02 |
| 2726 | Woburn | 3 | B02 |
| 2734 | Woburn | 3 | B02 |
| 2735 | Givskud | 3 | B02 |
| 2841 | Givskud | 3 | B02 |
| 2851 | Antwerp | 3 | B02 |
| 2861 | Howletts | 3 | B02 |
| 1954 | MKWC | 8 | B02 |
| 2106 | MKWC | 8 | B02 |
| 2271 | MKWC | 8 | B02 |
| 3087 | MKWC | 8 | B02 |
| 2295 | Marwell | 14 | B02 |
| 968 | Antwerp | 21 | B02 |
| 1942 | Chester | 193 | B02 |
| 2146 | Givskud | 193 | B02 |
| 2332 | Marwell | 193 | B02 |
| 2847 | Givskud | 193 | B02 |
| 1773 | Howletts | 308 | B02 |
| 1866 | Howletts | 308 | B02 |
| 2090 | Howletts | 308 | B02 |
| 2748 | Woburn | 308 | B02 |
| 2864 | Howletts | 308 | B02 |
| 1439 | Dvur Kralowe | 185 | B02 |

DNA samples were imported into the UK in 2019 (KWS non-CITES export permit no 0001909). Amplification focussed on the same left-hand 466 bp section of the control region assessed in previous studies (Faria et al., 2011; O'Donoghue et al., 2017) in order to produce directly comparable measures. Primers MT4 (Árnason et al., 1993) and B16168H (Simonsen et al., 1998) were used. PCR reactions were conducted in a volume of 6 µl with 1.5 µl of template DNA due to low concentration in the samples. The PCR protocol consisted of an initial denaturation of 5 minutes at 95 degrees followed by 32 cycles with 30s denaturation phase at 95°C, annealing at 48°C for 30s and 72°C for 30s, and a 10 minutes final extension at 72°C for. Amplicons were sequenced with Big Dye v3.1 in an ABI 3730 DNA Analyser at the Core Genomic Facility in Sheffield (UK).

Sequences were analysed in MEGA 7 (Kumar et al., 2016), and forward and reverse sequences were combined in a consensus sequence. Sequences were then aligned using the ClustalW algorithm in MEGA with default settings. As even experienced trackers can easily misidentify bongo dung at a rate of 30% (Faria et al., 2011), the control region was used to assign species identity (Bowkett et al., 2009); all consensus sequences were identified using BLAST (Altschul et al., 1990). Sequences identified as bongo were aligned and trimmed to a minimum length of 466 bp. In addition to the sequences retrieved here, I include sequences obtained in a previous study that sampled individuals from UK and Europe (Combe et al., 2018). Additional sequences assessed at Manchester Metropolitan University and not included in the mentioned study were added to the dataset, these consist of additional individuals from UK zoos, sampled using the same procedure described in Combe et al. (2018), Table 1 shows a list of all captive individuals included in the present study.

Differences among the sequences were searched in MEGA 7 and haplotypes were assigned relying on GENEBANK sequences for the two known haplotypes (B02 EU040246.1 and B01

EU040245.1; Faria et al., 2011). Haplotype richness and haplotype diversity in the sample were retrieved using the R package pegas (Paradis, 2010). I used the program POPART (Leigh and Bryant, 2015) to build and visualize a network of bongo haplotypes and to calculate nucleotide diversity (π). Moreover, I include Tajima's D (Tajima, 1989), a common index of genetic diversity that indicates whether a population is expanding.

## Results

I collected 28 samples in the Aberdares in 2017, 31 in 2018 and 24 in 2018 in Maasai Mau. However, amplification of the targeted segment of the control region was only successful in 37 wild samples, thus resulting in a much lower than expected sample size, with 3 amplified samples from Maasai Mau, and 34 from Aberdares. Of these, 10 were attributed to waterbuck, all found in the Aberdares, consistent with issue of sign misidentification highlighted by Faria (2011).

I found the same two haplotypes (B01 and B02, GENBANK accessions: EU04246 and EU04245) encountered in previous studies (Faria et al., 2011; O'Donoghue et al., 2017). Nucleotide diversity for both populations (Aberdares and Maasai Mau) was $\pi = 0.001$, while Tajima's D was 0.9 for the combined wild samples. The 22 samples from the Aberdares analysed in this study show a measure for Tajima's D of 0.9 with $\pi = 0.0012$. Although the value for of Tajima's D was not significant, I compared it with the value calculated from the 76 samples in the Faria et al. (2011) study, which show a value of 1.67 and 0.0014 for Tajima's D and π respectively. The two areas differ in haplotype diversity: zero in Maasai Mau, where only B01 is present in the three samples, and 0.48 in the Aberdares, where both haplotypes are present, with 15 samples assigned to B02 and eight to B01. In order to compare these results to those of a common antelope, the measures were also calculated for the 10 waterbuck samples, where five different haplotypes with a nucleotide and haplotype diversity of 0.02 and 0.76 respectively were found. Figure 2 shows a TCS haplotype network of the wild bongo samples compared to one developed for the waterbuck bycatch sample.

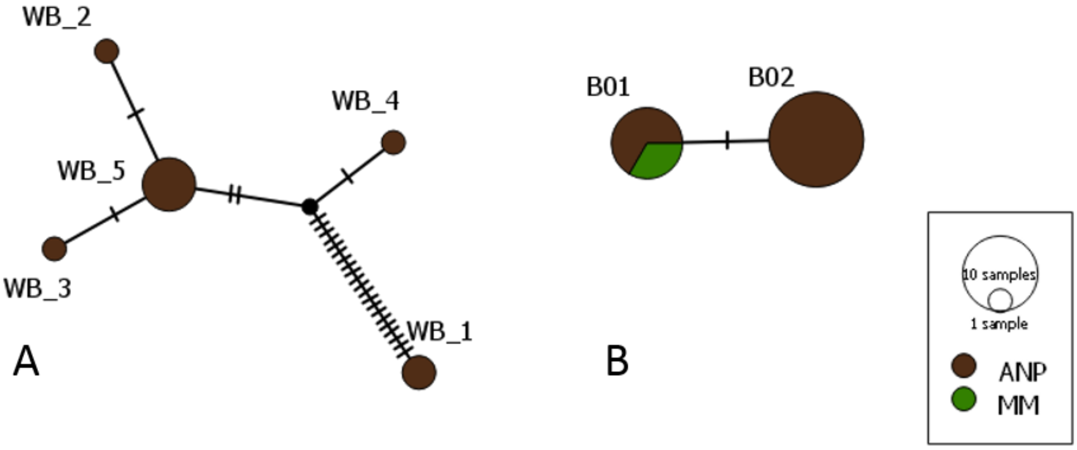

*Figure 2: TCS Haplotype network computed in PopArt. A) The network of the common waterbuck samples (N= 10) in the Aberdares. B) Network of mountain bongo samples (N= 25) from Maasai Mau (MM) and Aberdares (ANP).*

The reconstruction of the matrilines from the international studbook found that of the 34 females originally collected for the captive breeding programme, only 10 are still represented in living individuals globally (Figure 3).

My sample of captive individuals from Kenya (N = 14), combined with previous data concerning individuals from Europe and the UK (Combe et al., 2018, N = 31), covers six of the 10 extant matrilines (Figure 4). The sample represents 391 individuals of the 566 living animals that could be assigned to a lineage. Moreover, my sample includes 9 individuals from MKWC that could not be assigned to a matriline. Table 1 shows that all sequences from captive individuals in this study are of a single haplotype, B02. Table 1 also includes an individual sampled in O’Donoghue (2017), which belongs to a matriline not covered here. This individual shows the same haplotype.

## Discussion

My analysis aimed to compare the genetic diversity in wild and captive bongo. Samples were taken from the descendants of the major founding matrilines still extant in the captive population, both in Europe and in Kenya, as the latter is the most likely source of individuals for future reintroductions. My results suggest very low genetic diversity in captivity, with a single haplotype (B02) represented, consistent with previous findings (O’Donoghue et al., 2017). The results also show that the mtDNA in captive populations from European facilities and MKWC is representative of what is found in remnant populations in the wild. Thus, reintroduction for the establishment of new populations, and restocking of extant ones, should be seen as feasible with low risk of outbreeding depression due to the introduction of novel mitochondrial genomes. Moreover, with less than 40 individuals left in these remnant populations, and populations in Mt. Kenya and Eburu extinct or non-functional (Chapter 3) there is a clear need for demographic augmentation of bongo in the wild with both females and males.

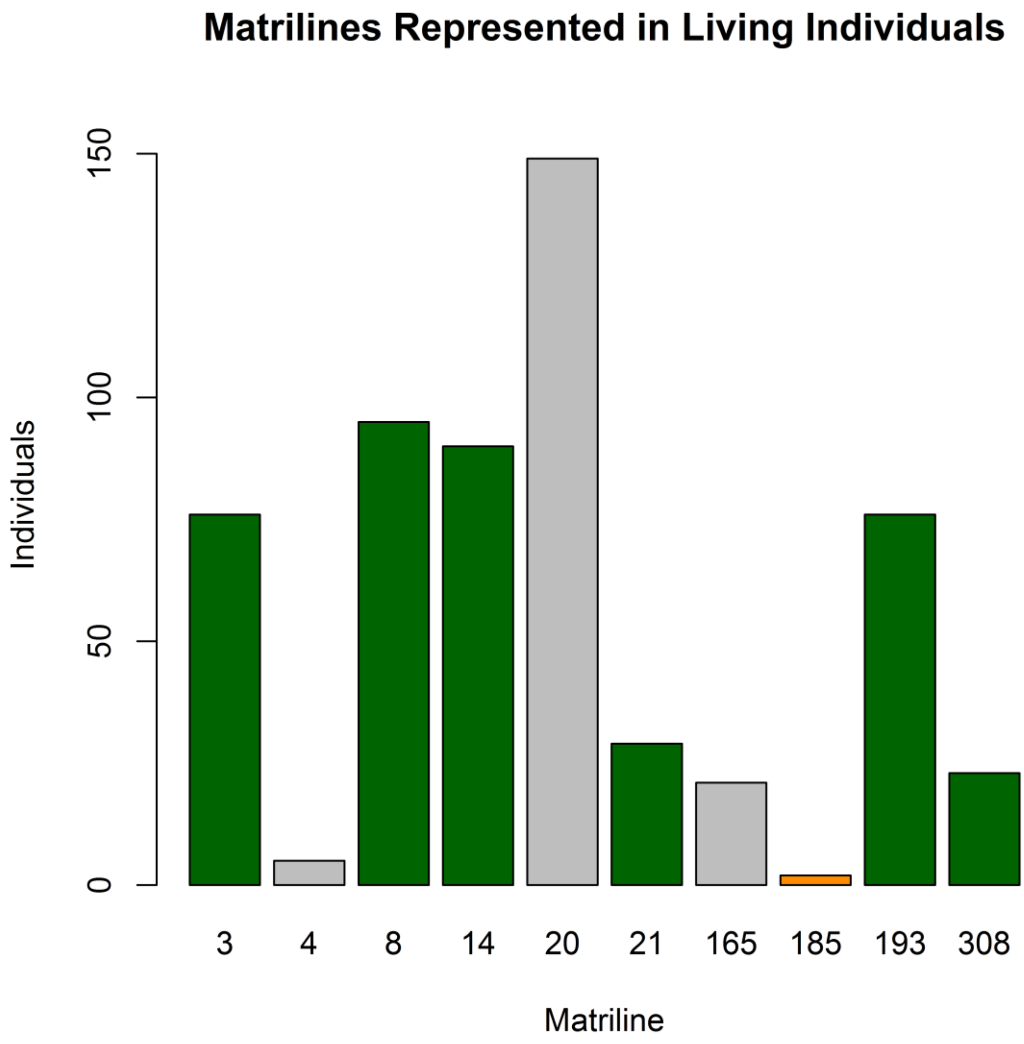

*Figure 3: Matrilines represented in the living bongo captive population. Matrilines are named using the studbook ID of founder females. Matrilines sampled within this study are highlighted in green. Orange indicates the matriline sampled in previous study (O'Donoghue et al., 2017).*

The risk of outbreeding depression in using captive individuals is a major limitation in genetic rescue attempts and reintroduction in general (Ralls et al., 2018). While these concerns are justified in case of individuals of unknown origin (Banes et al., 2016), the situation of mountain bongo captive population is the opposite. The captive population was founded with individuals from within the extant range and my results show that the captive population share genetic diversity with both breeding wild populations. Therefore, as a result of previous findings (Faria et al., 2011; O'Donoghue et al., 2017; Svengren et al., 2017) and the ones presented here, there appears to be no genetic risks of reintroduction using captive bred individuals.

The wild samples analysed here show an extremely low genetic diversity consisting of only two haplotypes. However, although limited (N = 3), samples collected from Maasai Mau all pertain to the rarest of the two haplotypes found in the Aberdares (B01). This information reinforces the importance of the Maasai Mau population  for the long-term conservation of this antelope- it is being the second largest population (Chapter 3) and it provides suitable habitat (Chapter 4). Considering that the Maasai Mau population may support a different representation of the known haplotypes to the Aberdares suggests that it may be beneficial to facilitate the gene flow between these areas. However, due to the lack of contiguity (Figure 1), assisted gene flow would be required (Frankham, 2010).

Assisted gene flow relies on translocating individuals among wild fragmented populations in order to permit levels of gene flow otherwise impossible to achieve without human intervention (Frankham et al., 2017). Increasing gene flow among populations is useful and can limit or delay the accumulation of inbreeding in small and fragmented populations (Frankham, 2015). In the case of mountain bongo it should not be seen as a genetic rescue attempt, as the two largest populations are breeding and, although no long-term data are available, they both appear to be growing (Chapter 3). Therefore, both extant and future restored populations of bongo would benefit from a management aimed at facilitating and assisting in maximising gene flow among them. Increased gene flow would not only increase the genetic variation, but also permit a higher adaptive potential in face of future challenges species may encounter (Aitken and Whitlock, 2013).

The extremely low diversity found in mountain bongo remnant wild population, and in captivity, is a reason for concern. Nevertheless, the findings from current and previous research (Faria et al., 2011) hint at the possibility that the low genetic diversity in mountain bongo may come from it being a marginal population of a wide-ranging species, the other subspecies being the Lowland bongo (*Tragelaphus eurycerus eurycerus*), spanning from west Africa throughout all central Africa (Elkan and Smith, 2013). Marginal populations are expected to show a lower genetic variation compared to their core counterparts (Eckert et al., 2008). Therefore, it is possible that even at the time of the establishment of the captive population, when a larger population resided in the Kenyan highlands (Kingdon, 1982), the genetic diversity was already low. Subsequent declines likely worsened this situation, and additional analysis of museum specimens and lowland bongo samples can help in better understanding the demographic history and elucidate the reasons for the extremely low genetic diversity of both wild and captive populations.

Due to the low diversity found at mitochondrial level, the use of nuclear markers or genomic tools would help in better assessing the variation present in both wild and captive populations, and it would help in understanding the relationship between these. Additional research is needed using nuclear markers (e.g. microsatellites or SNPs) to identify the best candidate captive lineages for maximising the impact of reintroduction by improving genetic variation in the wild. Nevertheless, the information presented here provides a useful insight into the fact that the wild and captive populations are likely very close genetically, and therefore future reintroductions, aimed at genetic rescue or, as I advocate, demographic rescue, could rely on the captive stock with little controversy.

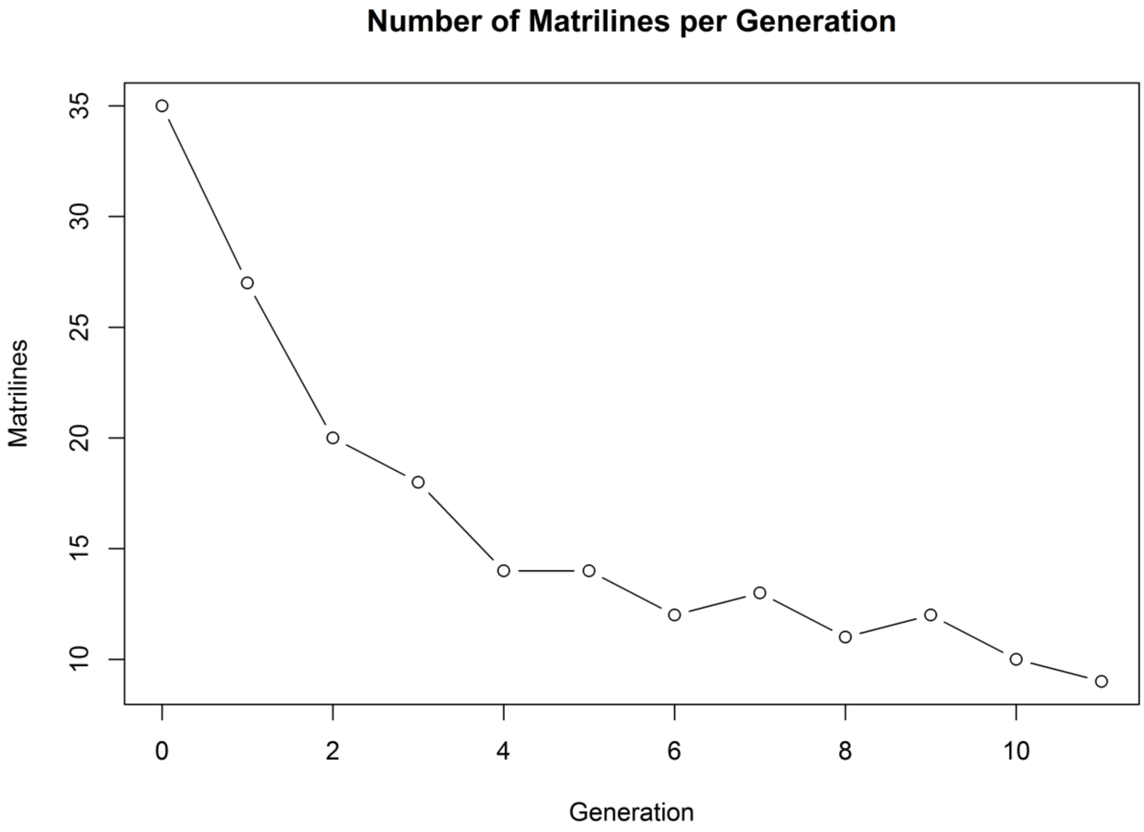

*Figure 4: Progressive decline of Matrilines represented in the captive population.*

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# Chapter 6:
# The Influence of Founder Relatedness in Captive Breeding Populations: an assessment relying on Simulated Pedigreed Populations and the Captive Population of a Critically Endangered Antelope.

### *Context in the Thesis*

*The focus of this last chapter is twofold. I assess through simulations the influence of founder relatedness in shaping the genetic makeup of living captive populations. I then rely on the well-recorded bongo studbook to understand whether the extremely low genetic diversity found in captivity could be due to an already deplete situation at the time of the foundation.*

### Abstract

Limiting inbreeding is one of the main goals when managing captive breeding populations, as its accumulation can lead to deleterious effects on the fitness of managed individuals, i.e. inbreeding depression. Moreover, inbreeding can lower the genetic variation of a population, thus reducing adaptive potential, which is of particular interest if an aim is to use captive individuals for reintroduction. The number of founders is likely to be a correlate of inbreeding; breeding programmes starting with larger population may show lower levels of inbreeding. However, measures of inbreeding currently employed in studbooks usually assume unrelated founders, which consequently affects estimates derived from pedigree data and may cause the often encountered discrepancies with measures of inbreeding based on genetic data. Here, I assess the influence that different levels of founder relatedness and population size have on the inbreeding of living individuals. I rely on simulated microsatellite-like loci in pedigrees of both simulated populations and mountain bongo (*Tragelaphus eurycerus isaaci*), a critically endangered antelope characterised by low genetic diversity. The long-term conservation of this antelope depends on a successful captive breeding programme. I then compare the results from the bongo pedigree with empirical measures derived from genotyped individuals in the European captive population. Results indicate that founder relatedness is more influential than size in determining measures of inbreeding in the living population, and the current inbreeding seen in mountain bongo is similar to that of an inbred simulated founder population. My findings can help in expanding current understanding on the influence of founder relatedness in measuring inbreeding. Moreover, they can help explain the extremely low genetic diversity encountered in captive bongo as resulting from an already depleted founder population rather than a shortcoming of the captive breeding programme.

## Introduction

Captive breeding is an important tool in the conservation of endangered species, as captive populations can reduce the probability of extinction and foster restoration or augmentation of wild populations (Conde et al., 2011). Nonetheless, the success of captive breeding depends on maintaining a high genetic variation, as it allows reintroduced individuals to adapt and evolve in response to environmental changes (Philippart, 1995; Frankham, 2005). Usually the foundation of a captive population creates a genetic bottleneck event, where typically only a portion of the genetic variation found in the wild is transferred to captivity, which can also lead to change in allele frequencies (Frankham et al., 1999; Ballou et al., 2010). Therefore, an important aim of captive breeding is to maximise retention of the genetic variation originally present in the founders (Lacy, 1989; Ballou et al., 2010).

A common problem in maintaining a captive population’s viability is inbreeding depression. Inbreeding is defined as the probability of the same copy of an allele being inherited by an individual from both parents due to a common ancestry (i.e. Identity by descent or IBD; Kardos et al., 2015). Inbreeding can lead to adverse effects by increasing the frequency of homozygosity of deleterious alleles and reducing overall heterozygosity with a direct impact on life history traits that can lead to a reduction in fitness, thus hampering the success of a captive breeding programme (Laikre, 1999). Hence, while retaining genetic variation, managers of captive populations must also minimize the occurrence of inbreeding (Hedrick and Kalinowski, 2000; Hedrick and Garcia-Dorado, 2016).

While inbreeding may hamper the viability of a captive population, allelic richness (i.e. the number of alleles found at a locus) has a direct effect on the adaptive potential of populations (Willoughby et al., 2015). Captive breeding can cause allelic richness to diminish, as a result of random loss through genetic drift (Philippart, 1995), or adaptation to captivity (Williams and Hoffman, 2009; Willoughby et al., 2017). Allelic richness can be estimated using neutral markers, where a high variation is considered indicative of overall genomic variation (Hedrick, 2001). Selection can cause a loss of alleles at neutral loci due to selective sweeps, which can result in genetic draft (Neher, 2013). The loss of alleles, and the associated loss of adaptive variation in captive populations, can hamper the success of reintroduction programmes, which is one of the ultimate aims of conservation breeding (Kraaijeveld-Smit et al., 2006). Therefore, captive breeding must limit the loss of genetic variation in managed populations.

Pedigrees, which in captive breeding are usually derived from studbooks, are the principal tool for maintaining and monitoring the genetic diversity derived from founders, and for avoiding inbreeding in captive populations (Princée, 2016a). Pedigrees estimate the relationship of each individual to others in the population (i.e. kinship). Prioritising individuals with the lowest kinship for breeding, referred to as Mean Kinship approach, is the most efficient way of retaining genetic variation while minimizing inbreeding (Willoughby et al., 2015). Moreover, in a pedigreed population we can measure the inbreeding coefficient of each individual, as the probability of its genome having identical alleles due to common ancestry links to its genealogy (Wright, 1965). However, this statistical approach means that individuals with similar genealogy will be assigned similar inbreeding coefficients, even though the actual alleles they inherited through their genealogies may be different (Hill and Weir, 2011). Furthermore, the reliance on information recorded in the pedigree comes with challenges: the record of individuals may be incomplete, paternities may have been wrongly assigned, or the full genealogy of individuals may have to be reconstructed *a posteriori* due to lack of a complete pedigree since the beginning of the captive breeding programme (Oliehoek and Bijma, 2009). Hence, although pedigrees can provide valuable information on the probability of an individual's genome being in part IBD, this measure may not reflect the reality found in the genome.

One of the assumptions of pedigree-based measures is non-relatedness of founders, which is unrealistic for most species as most wild populations show some background inbreeding (Crnokrak and Roff, 1999). Although the influence of this assumption on decision making and management of captive bred populations has been found to be negligible beyond the fifth generation (Rudnick and Lacy, 2008), it nonetheless affects the estimate of the magnitude of the inbreeding in a captive population by providing overly optimistic estimates (Hogg et al., 2018). Therefore, although knowledge of founder relatedness may not change the course of a captive breeding programme, understanding the influence of this assumption when calculating inbreeding in the living population is relevant for managers (Hogg et al., 2018). Thus, an estimate of accumulated inbreeding in a population that accounts for founder relatedness is important to assess whether a particular phenomenon in the population is due to inbreeding.

Alternative measures of inbreeding that rely on the heterozygosity found in genetic and genomic neutral markers (e.g. microsatellites, single nucleotide polymorphisms) have been employed to improve the accuracy of estimates of pedigree parameters in captive populations (Wang, 2014; Kardos et al., 2015). These marker-based approaches quantify inbreeding using the excess of homozygotes at target loci compared to that expected in an ideal scenario (i.e. Hardy-Weinberg equilibrium), as a lower heterozygosity could derive from a higher proportion of alleles being inherited due to IBD (Wright, 1965), thus relying on homozygosity as a proxy for inbreeding. Marker-based estimates of inbreeding are often in disagreement with measures from the pedigree (Wang, 2016). While limitations in sampling of both individuals and genetic markers exist, and although discrepancies may be due to errors in the pedigree, the underlying issue of non-relatedness of founders may also contribute to pedigree and marker-based genetic estimate disagreement. Hence, even when using marker-based approaches, the influence of founder relatedness is rarely investigated.

We know from previous research that founders have a strong influence on the living captive population, with the size of the founder population being a significant factor in determining inbreeding (Ralls and Ballou, 1986; Witzenberger and Hochkirch, 2011). Populations initiated with a higher number of founders show lower inbreeding compared to those started with few founders (Witzenberger and Hochkirch, 2011). However, the influence of the size of the founding population may be confounded by the genetic relatedness of founders. A limited number of founders collected from a single location may present a higher inbreeding state compared to multiple founders collected across the range. In captive breeding, it is common for founders to be representative of a fraction of the species range, both from a geographical and a genetic perspective (Hogg et al., 2019). A larger founding population may be advantageous compared to a small one due to an inherent lower relatedness. Therefore, taking into account the influence of founder relatedness along with population size is relevant for all those species that depend on captive breeding for their survival.

The mountain bongo (*Tragelaphus eurycerus isaaci*) is a critically endangered antelope endemic to Kenya (IUCN SSC Antelope Specialist Group, 2016), with a wild population of less than 50 individuals (Chapter 3) and low genetic diversity (Chapter 5; Faria et al., 2011). There is a successful international breeding programme which consists of over 700 captive individuals (Bosley, 2016), although even this population shows low levels of genetic diversity (Chapter 5; O'Donoghue et al., 2017). The bongo is an ideal candidate to investigate the influence of founder relatedness on the level of inbreeding as its global studbook (i.e. pedigree) is well documented; this alleviates discrepancies resulting from poor or erroneous record keeping. Besides, founder number is documented (Bosley, 2016), which facilitates accurate estimation of founder relatedness in captive pedigrees (Hogg et al., 2015). Of all the species that are currently managed in captive breeding programs for conservation, ungulates are known to be particularly susceptible to the occurrence of inbreeding depression (Ballou and Ralls, 1982). Hence, for the long term conservation of these species, which depends in part on captive breeding success (Hoffmann et al., 2015; Newby et al., 2016), it is important to have a better understanding of how founder population relatedness may be influencing the magnitude of inbreeding found in the living population.

In this study I will investigate the influence that inbred founders may have on genetic variation in a captive breeding program. i) I will assess the impact that different levels of inbreeding in the founding population has on inbreeding and allelic richness in simulated pedigrees, ii) I compare this to the influence of the size of founder population, and ii) I will compare results from simulated genotypes in the bongo pedigree with empirical measures from genotyped individuals within the captive population.

## Methods

### *Simulated Pedigreed Populations*

I simulate multiple pedigreed populations of a species under captive breeding conditions, with different settings for the founding individuals. These differ in their size and relatedness in order to assess and compare the influence of these parameters on the inbreeding and allelic richness of their descendants.

The simulated species has life history traits mimicking a polygynic ungulate with non-discrete generations, and that gives birth to a single offspring per generation. Individuals can breed once they reach maturity, here set at two, which is the age at first calving for bongo (Bosley, 2016). The sex ratio of offspring is constrained at 0.5, while the mortality is simulated as dependent on age with maximum age 10. Mortality is set at 0.4 before individuals reach maturity (age 0 – 2), 0.1 (age 3 – 7), mortality then increases to 1 at 10 years, details are shown in Table 1. Although most large ungulates can live beyond the age of 10, and still be fecund, here I use this threshold to exclude individuals from the pedigree in order to limit the size of populations in order to simplify calculation. Simulations were run for 20 generations (Mucha and Komen, 2016). An additional parameter included in the simulation refers to the average generation time, here set at two as the time needed for a female to become mature and produce individuals of the next generation, following the maternal rule in assigning generations (Princée, 2016b). Without such parameter, the 20 simulations would represent 20 years of breeding rather than the actual time it would take 20 generations to occur. Without such a parameter, founders would still be directly contributing to the population even in late generations, providing erroneous estimates of inbreeding.

*Table 1: Life history traits of the simulated species, the values aim to mimic a large ungulate, with values for breeding age retrieved from the bongo studbook (Bosley, 2016). Generation Time is set at 2 following the maternal approach, as I consider the minimum age here simulated for a female to produce the next generation. The age dependant mortality is adapted from a population viability analysis based on bongo studbook (Princée, 2016c). The maximum age at 10 is arbitrary.*

| | |
|---|---|
| Sex Ratio at birth | 0.5 |
| Breeding Age | 2 |
| Breeding Availability | 0.8 |
| N of Offspring | 1 |
| Generation time | 2 |
| Mortality | |
| Age 0 - 2 | 0.4 |
| Age 3 - 8 | 0.1 |
| Age 8 | 0.6 |
| Age 9 | 0.8 |
| Age 10 | 1 |

In order to mimic the breeding strategy used in zoos, all simulated pedigrees follow Mean Kinship as a pairing approach. This is achieved by creating a kinship matrix for each generation of individuals using the function ‘kinship’ in the R package {kinship2} (Sinnwell and Therneau, 2019). In each generation, breeding females are paired with males with an equal or lower mean kinship. I assumed the probability of individuals being available to breed at 0.8, thus mimicking the common issue of optimal pairings not being always available in captive breeding programmes (Mucha and Komen, 2016).

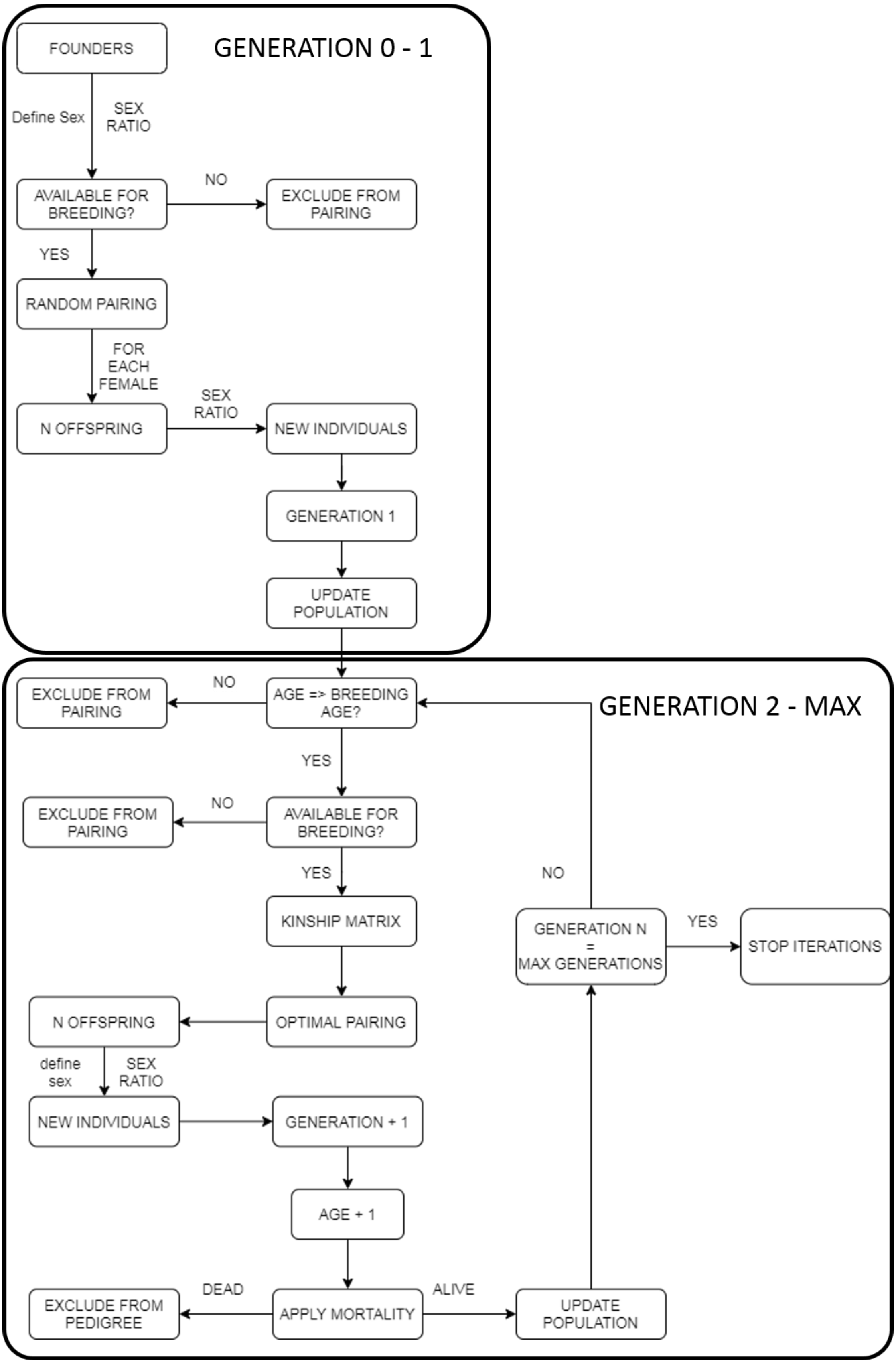

*Figure 1: A flowchart illustrating the process followed when simulating individuals in a pedigreed captive population.*

I simulate three levels of founder population size: 15 (minimum number of founders to minimize loss of genetic diversity; Witzenberger and Hochkirch, 2011), 50 and 100. The age of founders was simulated as a random value between breeding age and maximum age to mimic variable probability of founders contributing to the population for multiple generations (values shown in Table 1).

Pedigrees were simulated using custom code developed in R (R core team, 2019), and, following Mucha and Komen (2016), simulations were run for 20 generations and replicated 50 times for each level of founder population size (15,50,100), for a total of 150 pedigrees. Figure 1 illustrates the population simulation process.

### *Bongo Pedigree*

The bongo pedigree was retrieved from the international studbook relevant up to 2017 (courtesy of Nick Davis). The analysed pedigree consists of 3001 individuals from the global captive population including zoos, private ranches and reserves (Bosley, 2016). Individuals with incomplete ancestry were removed from the pedigree using the function 'tidyped' in the R package {visPedigree} (Luan, 2019). The resulting pedigree included 2492 individuals with full genealogy.

### *Genotype Simulation*

I simulated the inheritance of neutral microsatellite-like loci from founders along both the simulated pedigrees and bongo studbook following Mendelian rules, an approach comparable to Gene Drop (MacCluer et al., 1986). The number of microsatellite loci was set at 20, as this is a typical number of markers used in genetic analyses of captive populations (Wang, 2016), and I assumed an allelic richness of seven at each locus, which is the average found in wild mammals of conservation concern (Willoughby et al., 2015). In order to have different levels of founder relatedness, inbreeding was simulated as the probability of a founder to inherit an allele from a common randomly generated ancestral genotype. Therefore, the higher the inbreeding value the more likely founders would share the same genotype due to IBD. I rely on published observed heterozygosity values for mammals (Willoughby et al., 2015) in defining the inbreeding levels of the founders. Three levels of inbreeding were used in creating the founder genotypes: 0, representing the common assumption of no relatedness; 0.1 derived from the average heterozygosity found in wild mammals of conservation concern (Willoughby et al. 2015) and 0.3, to simulate a highly inbred founding population.

For each of the simulated pedigrees I calculate the inbreeding coefficient using genotypic data, relying on expected and observed heterozygosity, measured following F-statistics (Wright, 1965):

*Equation 1*

$$F = \frac{He - Ho}{He}$$

where *F* is the inbreeding coefficient, *He* is the expected heterozygosity under no inbreeding and *Ho* is the observed heterozygosity. Here, I use the proportion of heterozygote loci as a measure of heterozygosity. However, measures of inbreeding that rely on heterozygosity depend on a comparison of observed versus expected heterozygosity. Therefore, the reference heterozygosity used as the expected influences the measured inbreeding (Wang, 2014). Some measures use an expected heterozygosity of 1, as this is what would be found in a panmictic population (Falconer and MacKay, 1996). However, such a measure can be misleading, as it compares the heterozygosity found in the population to an ideal population, which would unrealistically increase the inbreeding coefficient; while we are interested in the real accumulation of homozygous loci compared to that found in the founder population (Wang, 2016). Therefore, *He* in this analysis refers to the proportion of heterozygote loci in simulated founders with 0 inbreeding, whereas *Ho* refers to the proportion of heterozygote loci in living descendants. Therefore, the measure I implement to calculate inbreeding will relate to the initial population, and realistically express the loss of heterozygosity along generations. In order to compare the inbreeding measure based on genotypes, I calculate the inbreeding coefficient from pedigree via path analysis (Wright, 1922) using the R package {pedigree} (Coster, 2012).

Results from the genotype simulations in the bongo pedigree were contrasted with a sample of 28 captive individuals from UK and European institutions genotyped at 19 loci with microsatellite markers (Combe et al. 2018). Although the samples were opportunistically collected across multiple institutions, 31 of the 38 founders that still contribute to the global living population are genetically represented in the 28 individuals included in this analysis. To retrieve *Ho* I calculated the proportion of heterozygote loci with the GENHET function in R (Coulon, 2010). Inbreeding was calculated for the empirical sample following Equation 1, using the heterozygosity of simulated loci in the unrelated founders (F = 0) as *He*.

In total, 450 simulations of genotypes were run, three different founder genotypes for each level of founder population size. Whereas, 50 genotype simulations were run along real the bongo pedigree. Genotypes simulated along the bongo real pedigree consisted of 19 loci to allow comparison to the empirical data retrieved from Combe et al. (2018).

*Allelic richness*

The assessment of allelic richness was conducted by retrieving the number of alleles still encountered in the living population at the end of the simulated 20 generations. Custom R code was developed to retrieve the information from the simulated pedigrees.

*Statistical Analysis*

The average inbreeding (both the pedigree and genotype measure), of the living individuals in each of the 150 simulated pedigreed population was retrieved using Equation 2.

*Equation 2*

$$\overline{F_j} = \frac{\sum_{i=1}^{N_j} F_{ij}}{N_j}$$

where $\overline{F_j}$ is the average inbreeding in population $j$, $N_j$ is the number of living individuals in population $j$ at the end of the simulated 20 generations. $F_{ij}$ is the inbreeding coefficient of the i-th living individual in population $j$. The same procedure was followed to retrieve the average allelic richness across the simulated loci using Equation 3.

*Equation 3*

$$\overline{A_J} = \frac{\sum_{i=1}^{N_{loci}} Aij}{N_{loci}}$$

Where $\bar{A_j}$ is the average number of alleles found in the living individuals of population $j$ across the assessed loci. $A_{ij}$ is the number of alleles at locus $i$ found across living individuals of population $j$. $N_{loci}$ is the number of assessed loci.

Retrieved values were included as dependent variables in separate model building processes with founder population size and inbreeding coefficient as single predictors and as covariates with and without interaction. The resulting four models for inbreeding and allelic richness were evaluated with an information theoretic approach through their AICc value (Doherty et al., 2012) using the package {AICcmodavg} (Mazerolle, 2019). Models that were more informative were interpreted to retain parameters that were relevant for the dependant variable analysed - inbreeding in the living population or its allelic richness. I then conduct a post hoc analysis, Tukey's HSD (Honest Significant Differences) test (Tukey, 1949; Abdi and Williams, 2010), to assess the influence of specific levels of each parameter (inbreeding and size of founder population), and their combination, on the average inbreeding and allelic richness in the living population. All statistical analysis was conducted in R (R core team, 2019).

## Results

### *Simulated pedigrees*

Results from the simulated pedigrees and simulated genotypes show that both inbreeding and size of founder population are relevant factors in shaping inbreeding and allelic richness of the living population. Level of inbreeding is best explained by a combination of founder relatedness and population size, as shown in the model selection shown in Table 2. The same is true for allelic richness, also shown in Table 2. However, when used as single predictors, inbreeding and population size of founders behave differently. Founder relatedness is more relevant in both analyses, but while it has similar relevance to population size in explaining allelic richness, it has a much larger importance than founding population size in explaining inbreeding. Size presents an $R^2$ of just 0.04 in predicting inbreeding, compared to 0.43 when the same parameter predicts allelic richness (Table 2). Thus, while inbreeding and population size of founders similarly shape allelic richness, the former is more important in predicting inbreeding in the living population. Nevertheless, these results show that when considered individually, neither parameter is capable of fully predicting inbreeding and allelic richness.

*Table 2: Model selection Table for Inbreeding and Allelic richness of living population depending on inbreeding of founders (F) and number of founders (N).*

| **Inbreeding** | | | | |
|---|---|---|---|---|
| Model | K | ΔAICc | Log Likelihood | $R^2$ |
| F + N + F*N | 10 | 0 | 864.41 | 0.92 |
| F + N | 6 | 6.21 | 857.15 | 0.91 |
| F | 4 | 196.58 | 759.91 | 0.86 |
| N | 4 | 1079.85 | 318.28 | 0.04 |
| **Allelic Richness** | | | | |
| Model | K | ΔAICc | Log Likelihood | $R^2$ |
| F+N+F*N | 10 | 0 | -155.06 | 0.92 |
| F+N | 6 | 48.18 | -183.3 | 0.91 |
| F | 4 | 807.84 | -565.18 | 0.48 |
| N | 4 | 856.83 | -589.68 | 0.43 |

Table 3 shows the results of the *post hoc* analysis (Tukey's HSD test) on the most informative models for allelic richness and inbreeding (Table 2). Given the same level of inbreeding in founders, a founding population of 100 does not significantly influence the inbreeding coefficient in the living population compared to a 50 founders. The same is found for allelic richness solely in the case where founders show no inbreeding. Results also show that the preservation of founder alleles is affected by their inbreeding, there is a reduction in alleles after the simulated 20 generations. In the living population there was no difference in average allelic richness the highly inbred population but with many founders and the small population with a few but outbred founders . The limited difference between founder populations of 50 and 100 is also found in the preservation of allelic richness.

As expected, the pedigree-based measure of inbreeding appears to be in agreement with the genotype derived measure only in case of non-related founders (F = 0; Wilcoxon test p > 0.05, Figure 2).

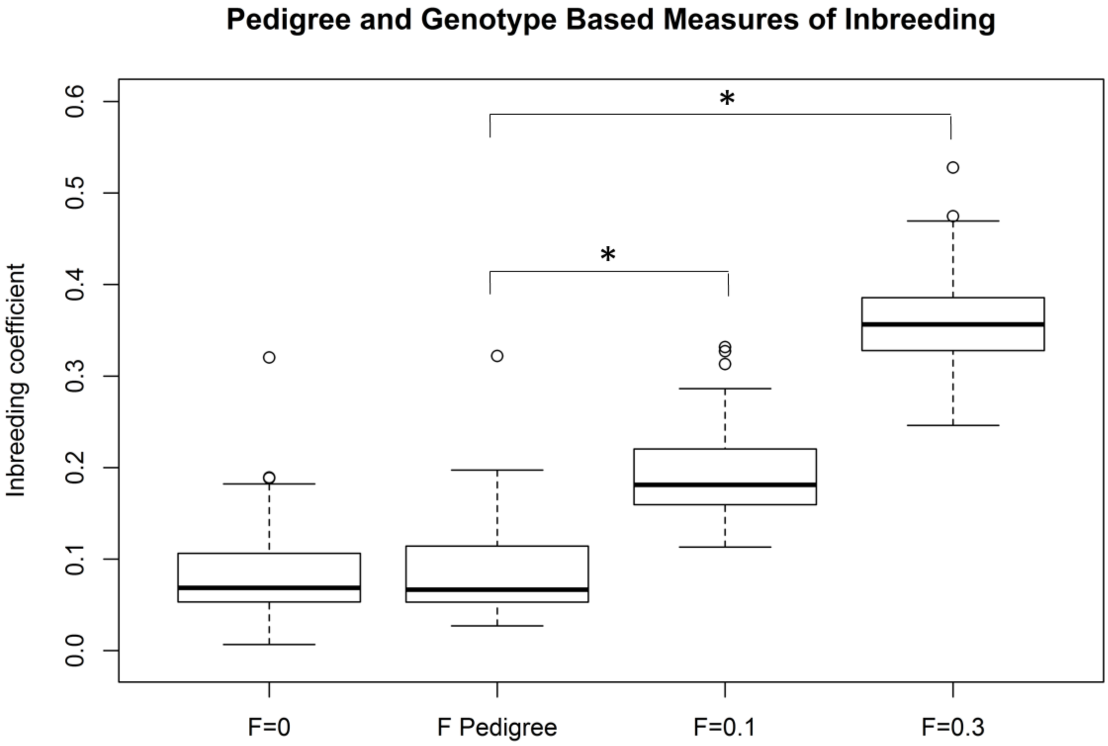

*Figure 2: Comparison of Pedigree based and Genotype based measure of Inbreeding in simulated pedigrees.*

*Bongo Pedigree*

As with the simulated pedigrees, the inbreeding coefficient measured with simulated genotypes in the bongo studbook is significantly different to the pedigree-based measure in all cases except for unrelated founders. The observed heterozygosity of the 28 individuals measured on real genotypes appears similar to the one found in the simulated genotypes with a highly inbred founding population (F = 0.3, Figure 3). The inbreeding of the 28 genotyped individuals was calculated with Equation 1 using the heterozygosity of simulated unrelated founders (*He* = 0.82). The difference between real and simulated inbreeding is significant in every instance except for simulated genotypes resulting from highly inbred founders (Wilcoxon test, p = 0.05; Figure 4).

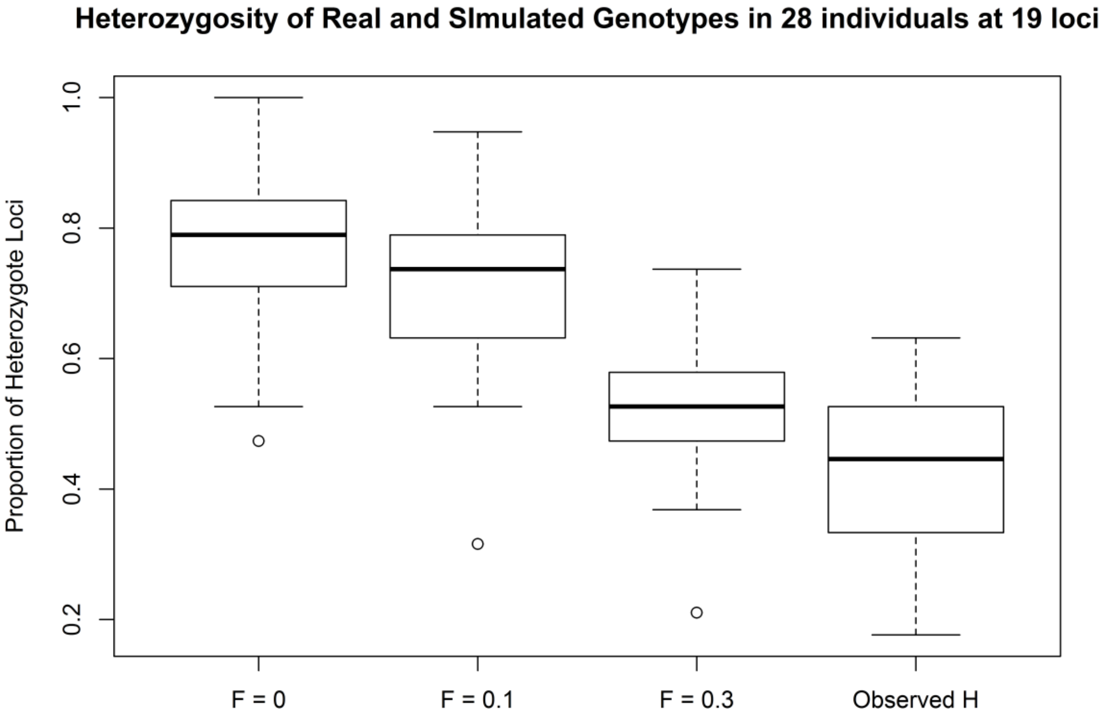

*Figure 3: Observed Heterozygosity in simulated and real bongo genotypes.*

*Table 3: Results of the post hoc analysis on the most informative models for inbreeding and allelic richness found in simulated living individuals (models are shown in Table 2). The analysis was conducted using Tuckey HSD test and the table shows results for the combined influence of inbreeding and size of the founder population. Difference indicates the difference in the mean of the value for inbreeding and allelic richness in living individuals. LCI = lower limit of the 95% confidence interval, UCI = upper limit of the 95 % confidence interval. Rows where differences are not significant are highlighted and in bold.*

| | ***Inbreeding*** | | | | ***Allelic Richness*** | | | |
|---|---|---|---|---|---|---|---|---|
| *Inbreeding : Size* | *Difference* | *LCI* | *UCI* | *p* | *Difference* | *LCI* | *UCI* | *p* |
| 0.1:15 - 0:15 | 0.10 | 0.08 | 0.12 | 0.00 | -0.90 | -1.12 | -0.69 | 0.00 |
| 0.3:15 - 0:15 | 0.26 | 0.23 | 0.28 | 0.00 | -2.13 | -2.35 | -1.92 | 0.00 |
| 0:50 - 0:15 | -0.06 | -0.09 | -0.04 | 0.00 | 1.27 | 1.06 | 1.49 | 0.00 |
| 0.1:50 - 0:15 | 0.04 | 0.02 | 0.07 | 0.00 | 0.61 | 0.40 | 0.83 | 0.00 |
| 0.3:50 - 0:15 | 0.23 | 0.20 | 0.25 | 0.00 | -0.90 | -1.11 | -0.68 | 0.00 |
| 0:100 - 0:15 | -0.07 | -0.10 | -0.05 | 0.00 | 1.44 | 1.22 | 1.65 | 0.00 |
| 0.1:100 - 0:15 | 0.04 | 0.02 | 0.06 | 0.00 | 1.12 | 0.91 | 1.34 | 0.00 |
| **0.3:100 - 0:15** | 0.21 | 0.19 | 0.23 | 0.00 | **-0.16** | **-0.37** | **0.06** | **0.34** |
| 0.3:15 - 0.1:15 | 0.16 | 0.13 | 0.18 | 0.00 | -1.23 | -1.45 | -1.02 | 0.00 |
| 0:50 - 0.1:15 | -0.16 | -0.19 | -0.14 | 0.00 | 2.17 | 1.96 | 2.39 | 0.00 |
| 0.1:50 - 0.1:15 | -0.06 | -0.08 | -0.03 | 0.00 | 1.51 | 1.30 | 1.73 | 0.00 |
| **0.3:50 - 0.1:15** | 0.13 | 0.10 | 0.15 | 0.00 | **0.01** | **-0.21** | **0.22** | **1.00** |
| 0:100 - 0.1:15 | -0.18 | -0.20 | -0.15 | 0.00 | 2.34 | 2.12 | 2.55 | 0.00 |
| 0.1:100 - 0.1:15 | -0.06 | -0.08 | -0.04 | 0.00 | 2.03 | 1.81 | 2.24 | 0.00 |
| 0.3:100 - 0.1:15 | 0.11 | 0.08 | 0.13 | 0.00 | 0.74 | 0.53 | 0.96 | 0.00 |
| 0:50 - 0.3:15 | -0.32 | -0.34 | -0.30 | 0.00 | 3.41 | 3.19 | 3.62 | 0.00 |
| 0.1:50 - 0.3:15 | -0.21 | -0.23 | -0.19 | 0.00 | 2.75 | 2.53 | 2.96 | 0.00 |
| 0.3:50 - 0.3:15 | -0.03 | -0.05 | -0.01 | 0.00 | 1.24 | 1.02 | 1.45 | 0.00 |
| 0:100 - 0.3:15 | -0.33 | -0.35 | -0.31 | 0.00 | 3.57 | 3.36 | 3.79 | 0.00 |
| 0.1:100 - 0.3:15 | -0.22 | -0.24 | -0.19 | 0.00 | 3.26 | 3.04 | 3.47 | 0.00 |
| 0.3:100 - 0.3:15 | -0.05 | -0.07 | -0.03 | 0.00 | 1.98 | 1.76 | 2.19 | 0.00 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 0.1:50 - 0:50 | 0.11 | 0.09 | 0.13 | 0.00 | -0.66 | -0.88 | -0.45 | 0.00 |
| 0.3:50 - 0:50 | 0.29 | 0.27 | 0.31 | 0.00 | -2.17 | -2.38 | -1.95 | 0.00 |
| **0:100 - 0:50** | **-0.01** | **-0.03** | **0.01** | **0.77** | **0.17** | **-0.05** | **0.38** | **0.28** |
| 0.1:100 - 0:50 | 0.10 | 0.08 | 0.13 | 0.00 | -0.15 | -0.36 | 0.07 | 0.44 |
| 0.3:100 - 0:50 | 0.27 | 0.25 | 0.29 | 0.00 | -1.43 | -1.65 | -1.22 | 0.00 |
| 0.3:50 - 0.1:50 | 0.18 | 0.16 | 0.20 | 0.00 | -1.51 | -1.72 | -1.29 | 0.00 |
| 0:100 - 0.1:50 | -0.12 | -0.14 | -0.10 | 0.00 | 0.83 | 0.61 | 1.04 | 0.00 |
| **0.1:100 - 0.1:50** | **0.00** | **-0.03** | **0.02** | **1.00** | 0.51 | 0.30 | 0.73 | 0.00 |
| 0.3:100 - 0.1:50 | 0.16 | 0.14 | 0.19 | 0.00 | -0.77 | -0.99 | -0.55 | 0.00 |
| 0:100 - 0.3:50 | -0.30 | -0.32 | -0.28 | 0.00 | 2.33 | 2.12 | 2.55 | 0.00 |
| 0.1:100 - 0.3:50 | -0.19 | -0.21 | -0.16 | 0.00 | 2.02 | 1.80 | 2.23 | 0.00 |
| **0.3:100 - 0.3:50** | **-0.02** | **-0.04** | **0.00** | **0.17** | 0.74 | 0.52 | 0.95 | 0.00 |
| 0.1:100 - 0:100 | 0.12 | 0.09 | 0.14 | 0.00 | -0.31 | -0.53 | -0.10 | 0.00 |
| 0.3:100 - 0:100 | 0.28 | 0.26 | 0.31 | 0.00 | -1.60 | -1.81 | -1.38 | 0.00 |
| 0.3:100 - 0.1:100 | 0.17 | 0.14 | 0.19 | 0.00 | -1.28 | -1.50 | -1.07 | 0.00 |

## Discussion

The results show that both size and inbreeding of the founder population influence the level of inbreeding of the living population and its allelic richness. Founder relatedness influences the inbreeding of the living population more than its size, while the latter appears more relevant in the retention of founder alleles. Nevertheless, results indicate that both parameters need to be considered to fully explain the level of inbreeding found in the living population. In addition, the simulation of genotypes in the real bongo pedigree indicate that the inbreeding we find in the current population may be explained by a higher than usually assumed relatedness in the founders of this captive breeding programme.

These findings provide additional evidence of the influence of founder population size on the inbreeding of living individuals, where a large founder population slows the accumulation of inbreeding (Witzenberger and Hochkirch, 2011). However, by including the inbreeding of founders in the analysis it is clear that the number of founders cannot fully explain the genetic make-up of living individuals. This gives a novel insight into the role of founder population size, as it is likely that what is actually affecting the inbreeding of current populations is the relatedness of their founders, which is likely to be higher in programmes initiated with fewer founders (Ralls and Ballou, 1986). Thus, results suggest that influence on the inbreeding of living individuals attributed to size of founder population might be in fact due to their relatedness.

My results show that the size of founder population is important in retaining genetic variation. When size is used as a single parameter in a linear model, it is more informative in predicting the allelic richness of the living population rather than its inbreeding. This indicates that more founders will result in more alleles found in the population of origin being conserved, which is key in future adaptability and reintroduction success (Kraaijeveld-Smit et al., 2006; Willoughby et al., 2015). It also allows the captive population to be successful in terms of conserving important variation found in the wild (Leus et al., 2011). However, *post hoc* analysis shows that founder relatedness is relevant in shaping allelic richness too, as the inbreeding of founders can hamper the ability of population size to retain alleles. For instance, results presented in Table 3 show that founding a captive population with 15 non-related founders would manage to retain the same average allelic richness as one founded with 100 highly inbred individuals. Therefore, my results show that a larger number of founders does not insure against loss of alleles, as the underlying issue of founder relatedness may limit the amount of genetic variation that is conserved.

Any simulated inbreeding level higher than zero causes discordances between the genotype measure and the pedigree-only estimate. However, for simulations with F = 0 (i.e. consistent with the assumption of non-relatedness of founders) no significant difference was observed between marker-based or pedigree-based F estimates. This indicates that a limited sample of polymorphic loci (similar to the one simulated here) still follow the pedigree measure, as long as the assumption of non-relatedness is true. However, even small deviation from the assumption causes the two measures to disagree, suggesting that discrepancies between pedigree-based and genotype-based estimates of genetic parameters may arise due to the genetic characteristics of founders in addition to limitations of markers (Wang, 2016). Moreover, the effect of recombination and linkage disequilibrium were not simulated here, these likely affect genetic structure, particularly in small populations with a pronounced founder effect (Kardos et al., 2015), and analyses including effects would improve the understanding of the role of founder relatedness. It also should be noted that in this analysis all individuals in the population were sampled, therefore the main limitation in marker base assessments might relate to the sampling of the population rather than the marker characteristics. Thus, although errors in the pedigree and limitations in sampling individuals are a major issue when calculating inbreeding, the acknowledgement of founder relatedness can help in explaining the situation of living captive populations.

The comparison of results from simulated genotypes in the complete captive pedigree of the mountain bongo provides further evidence for the likely influence of founder relatedness. Results from the simulations and from an empirical study based on a representative sample of captive individuals show that the inbreeding, as calculated at 19 loci, is close to that of a simulated population of inbred founders (Figure 4). Errors in the compiling of the pedigree, recombination, and linkage disequilibrium may account for some of the discrepancies, but my results show evidence of a non-negligible effect of the founders. Considering the history of the provisioning of wild bongo for the captive populations this was a likely outcome: all individuals were sourced from a single area (Aberdares in central Kenya), and the wild population had already suffered a decline due to a combination of rinderpest outbreaks, habitat loss and hunting pressure (Elkan and Smith, 2013). Hence, the individuals were deemed to have some level of inbreeding and violate the non-relatedness assumption. The findings of this analysis are in agreement with Hogg et al. (2019) on the importance of a carefully sourcing of individuals when founding a captive population. This was certainly not the case for the bongo as the population in the 1960’s and 1970s was already compromised in terms of its ability to provide suitable founders for a captive population. Furthermore, the loss of the majority of matrilines in the first four generations, from 35 to 12 (Figure 5), likely limited the ability of the captive breeding program to maintain higher genetic diversity.

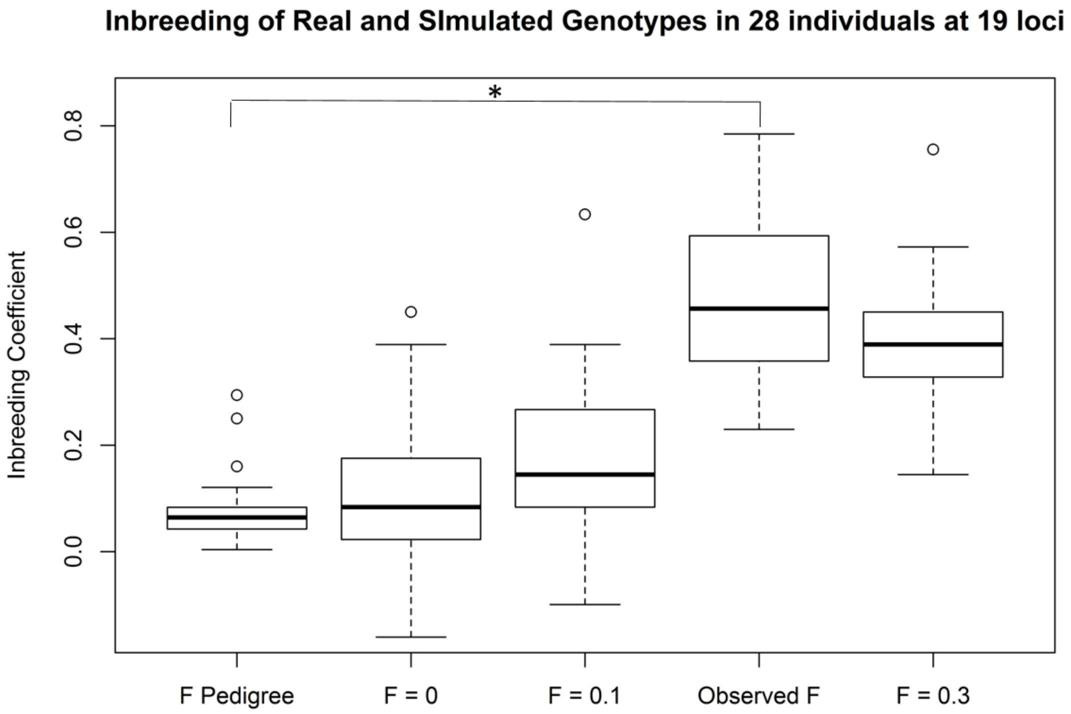

*Figure 4: Inbreeding coefficient in simulated and real genotypes. Pairwise Wilcoxon tests were run to assess significance of differences. In all cases except for the simulated genotypes originating from highly inbred founders (F = 0.3) a significant difference was found.*

A limit of the approach here is the assumption that heterozygosity in the living population, or a lack of it, are indicative of inbreeding. This assumption has been challenged, as strong correlations between inbreeding and heterozygosity are not common (Balloux et al., 2004). Nevertheless, my results indicate that the genetic situation of founders, be it due to inbreeding or to an inherent low diversity, influences the genetics of the living population. Therefore, the situation we encounter in captivity is likely due to an already low level of heterozygosity in founders. Further analysis aimed at reconstructing bongo demographic history should be implemented to understand whether the suspected compromised situation was due to inbreeding or to an inherent low genetic variation.

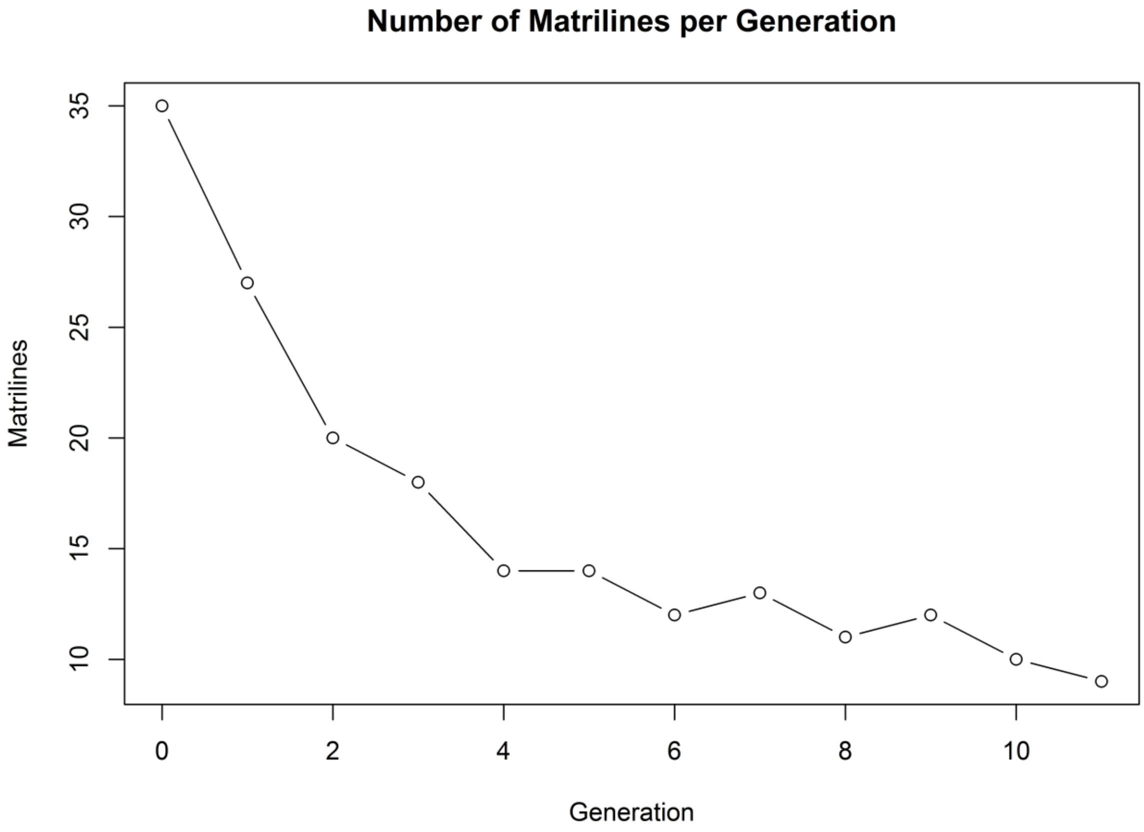

*Figure 5: Progressive decline of Matrilines represented in the captive population.*

The captive population of bongos resulted from the collection of over 70 wild individuals, which, according to results here and elsewhere (Witzenberger and Hochkirch, 2011) may have limited inbreeding accumulation. However, if genetic diversity and heterozygosity were already low at the time of the founders, the amount of genetic diversity retained is also likely low. This conclusion agrees with recent findings showing an extremely low genetic diversity, in particular within mitochondrial DNA, in the captive population (Chapter 5; O'Donoghue et al., 2017). Results from the simulated genotypes using the bongo pedigree may suggest that current genetic predicament of the captive bongos is an indication of a compromised founder population rather than shortcomings in the international breeding programme. Therefore, pairing simulated and real pedigrees, and simulated and real genotypes, provides a valuable insight into the influence of founder relatedness on living populations currently managed in conservation breeding programmes.

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# Chapter 7:
# General Discussion

## Introduction

The global loss of biodiversity calls for conservation actions to limit the impact of what is considered the sixth mass extinction (Ceballos et al., 2017). These actions rely on information regarding demography, habitat and genetics of populations to be effective and efficient in the preservation of species (Mills, 2013). This important information that managers and conservationists rely on comes from research and monitoring programmes. This is particularly relevant for species and populations that are in a critical situation due to their small population size and a fragmented distribution. In light of this, the project presented here investigates the demographics, habitat associations, and genetics of Mountain bongo (*Tragelaphus eurycerus isaaci*), a Critically Endangered antelope endemic to the highlands of Kenya, with the aim of providing information for its effective conservation.

## Key Findings from each Chapter

### *Chapter 2*

The development of an identification system is the main result from this chapter. The system relies on visual features that are identifiable in both daylight and nighttime camera trap images: facial markings, stripe pattern, and horn state. The system is both informative and reliable among naïve observers, with a value for Light's Kappa of 0.64 (where 0 would mean no agreement and 1 full agreement), and I anticipate a higher reliability in the context of expert practitioners and field-workers. The use of an easy and accessible user based system will allow the population monitoring of this antelope to proceed and managers could implement the same method to monitor individuals post-release in future reintroductions. Additionally, the chapter developed a quantitative tool for the efficient discrimination of bongo from waterbuck through measures of their tracks. I found a threshold value at which one can confidently assign tracks to bongo (track aspect ratio ≤ 1.2).

### *Chapter 3*

The main result presented in this chapter is the first insight into the demographics of the remnant populations of bongo in the wild. I combined the ID system developed in Chapter 2 with the extensive camera trap footage monitoring activity on the four remnant populations of Aberdares, Maasai Mau, Eburu and Mt. Kenya conducted by the Bongo Surveillance Project (BSP). This allowed the application of Mark-Recapture methods, thus extracting hitherto inaccessible information contained in BSP footage. Previously only information about the persistence of these populations was available, while thanks to results presented in this chapter, their size, structure, and dynamics are now known. Results indicate a critical situation for both Eburu and Mt. Kenya (where a single individual was identified at each, a male and a female respectively), while adults of both sexes and calves are systematically encountered only in the populations of Maasai Mau and Aberdares. Population size estimates of 39.58 (CI 29.2 – 49.8) in the entire range are in agreement with the lowest IUCN estimate of less than 50 individuals. Furthermore, survivorship estimates specific to sex and age (≤ 2 years, and mature) were retrieved, these will provide a solid base for future decision-making and allow the assessment of the short-term viability of these populations. The retrieval of estimates for survivorship not only provides valuable information for the managers, but also sheds light on a usually difficult parameter to estimate in forest species, proving the efficacy of a strategic placement of the camera traps at points of activity (i.e. salt licks).

### *Chapter 4*

The knowledge gathered on the demographics of the remnant populations forced the rethink of our understanding of where suitable areas for bongo may be, as of the four areas, breeding herds are only found in Maasai Mau and the Aberdares. While a distribution model for bongo already existed thanks to the work of Estes et al. (2011), some of the areas where bongo were encountered during previous surveys (Estes et al., 2008) have since failed to provide evidence of persistence of the antelope, and it is likely bongos in these areas are now extinct or at very low densities there. Therefore, the model presented in this chapter, while relying on fewer data compared to previous research, relies on presence points from areas known to host breeding populations, thus making its predictions of suitable habitat throughout the current bongo range more reliable. This information will allow managers and conservationists to refine and direct their effort in the conservation of the remnant populations, and in locating promising areas for reintroduction. The chapter includes an analysis on the influence of disturbance on bongo presence. Disturbance, which in the surveyed areas consists mainly of selective logging, shows a negligible effect when included as a parameter in a predictive model, in accordance with previous research where selective logging does not significantly affect biodiversity (Edwards and Laurance, 2013).

*Chapter 5*

The assessment of the diversity found at mtDNA control region in two wild populations (Maasai Mau and Aberdares) and in captivity (Mount Kenya Wildlife Conservancy and European zoos) confirms previous results indicating an extremely low diversity. Only two haplotypes are found in the wild and only one of these is represented in the majority of matrilines within the captive breeding program. Nevertheless, while low genetic variation is not optimal for the long-term persistence of populations, the results indicate that there is limited, if any, risk of outbreeding depression in case of reintroducing captive individuals. Moreover, the analysis managed to highlight the relevance of Maasai Mau in the conservation genetics of bongo, as it harbours the least represented of haplotypes.

The use of both simulated populations and simulated genotypes has allowed investigating the influence of founder relatedness on both inbreeding coefficient and allelic richness in captive breeding programs. Results show that the inbreeding of founders influences the inbreeding found in the living population, while founder population size, usually considered a major predictor of inbreeding in living individuals (Witzenberger and Hochkirch, 2011), is in fact more important in maintaining allelic richness. The comparison of inbreeding measured through both simulated genotypes and real genotypes in the bongo pedigree has provided evidence that the bongo founder population, although consisting of over 70 individuals, likely showed low genetic variation, possibly due to inbreeding. Results thus suggest that at the time of the foundation of the captive population, bongo in the wild already showed depleted genetics. Therefore, in light of the results from the simulated populations, the low genetic variation found in captive bongo should be interpreted as being a consequence of an already compromised situation in the founder population rather than a shortcoming of the captive breeding program.

## Implications for Conservation and Management

While the project fulfilled its aim of gathering information for the effective conservation of Mountain bongo populations, both wild and captive, a common issue in conservation is that of the research – implementation gap (Knight et al., 2008; Sunderland et al., 2009). This gap affects in particular developing countries with limited access to peer-reviewed journals and other purely academic outlets (Gossa et al., 2015), which can lead to information gathered through research failing to become available and used by managers. In order to limit this situation, the project presented in this thesis was conducted in close collaboration with the ultimate authority in charge of the management of Mountain bongo in the wild, the Kenya Wildlife Service (KWS). This collaboration allowed us to reduce the research-implementation gap, as information gathered during all stages of the project was immediately shared with KWS. The tools developed within the project, presented in Chapter 2, and additional methods followed in the project have been included as an appendix in KWS national strategy and action plan for Mountain bongo (KWS, 2019). Moreover, the same methods, tools and results are being shared with the Bongo Surveillance Project (BSP), which conducts monitoring of the remnant populations throughout Kenya. Therefore, while research – implementation gap is a challenge faced by conservation biology, a close relationship with non-academic organizations working with the target species allows this gap to narrow and therefore information could readily be used for the design of conservation actions.

### *Monitoring and Protection of the Remnant Populations*

The first effective action in the conservation of the remnant populations of bongo is to reinforce the current monitoring effort. Monitoring is paramount for conservation at any level (Yoccoz et al., 2001) and without the monitoring conducted by BSP throughout the recent years, findings from this project would have been impossible to gather. However, the monitoring BSP conducts is of a surveillance nature, now, thanks to newly developed tools and baseline information on size and vital rates, this monitoring could evolve into more effective population monitoring (Nichols and Williams, 2006). Such a monitoring would allow the managers of the areas where remnant bongo populations are found to have a continuous flow of information not only on the persistence, as it is today, but also on the growth and viability of these populations. Following an adaptive approach in both the monitoring (Lindenmayer et al., 2011) and the management (Schwartz et al., 2012; Serrouya et al., 2019) of wild bongo can help avoid monitoring populations to extinction (Lindenmayer et al., 2013). Therefore, population monitoring is an immediate need for the effective conservation of the remnant populations.

The Aberdares have long been considered the stronghold of Mountain bongo (Kingdon, 1982; Elkan and Smith, 2013), and this view is confirmed here. Results from Chapter 3 indicate that this mountain range is home to the largest of the surveyed populations, although it appears all individuals pertain to a single herd inhabiting a specific area, the Salient (Figure 1). Other areas in the Aberdares where bongo were encountered as late as in the early 2000's (Estes et al., 2008) have failed to provide evidence of their presence in later surveys. Therefore, the focus to protect the remnant population in this area should be on protecting the Salient. However, results in Chapter 4 show that in the Aberdares areas of suitable habitat are limited and disconnected. Therefore, while it is here confirmed that the Aberdares are the most important area when it comes to population size, the long-term conservation of this antelope cannot depend on the protection of only this stronghold. Managers and conservationist must also prioritise other areas to preserve this antelope in the long-term.

The relevance of Maasai Mau for bongo conservation is a major finding of this project. This area is home to the second largest population, with results from Chapter 3 showing a breeding and growing population, although of very small size and likely consisting of a single herd. Moreover, while the area occupied by the resident bongo remains unknown, Maasai Mau appears to consist of mostly suitable habitat, as illustrated in Chapter 4, thus making it a candidate area for reintroduction. Furthermore, the finding of the least represented control region haplotype in the Maasai Mau samples found in Chapter 5 hints at the relevance of this area for the long-term genetic viability of wild bongo. Results thus show that the protection of Maasai Mau is paramount for the long-term conservation of Mountain bongo in the wild.

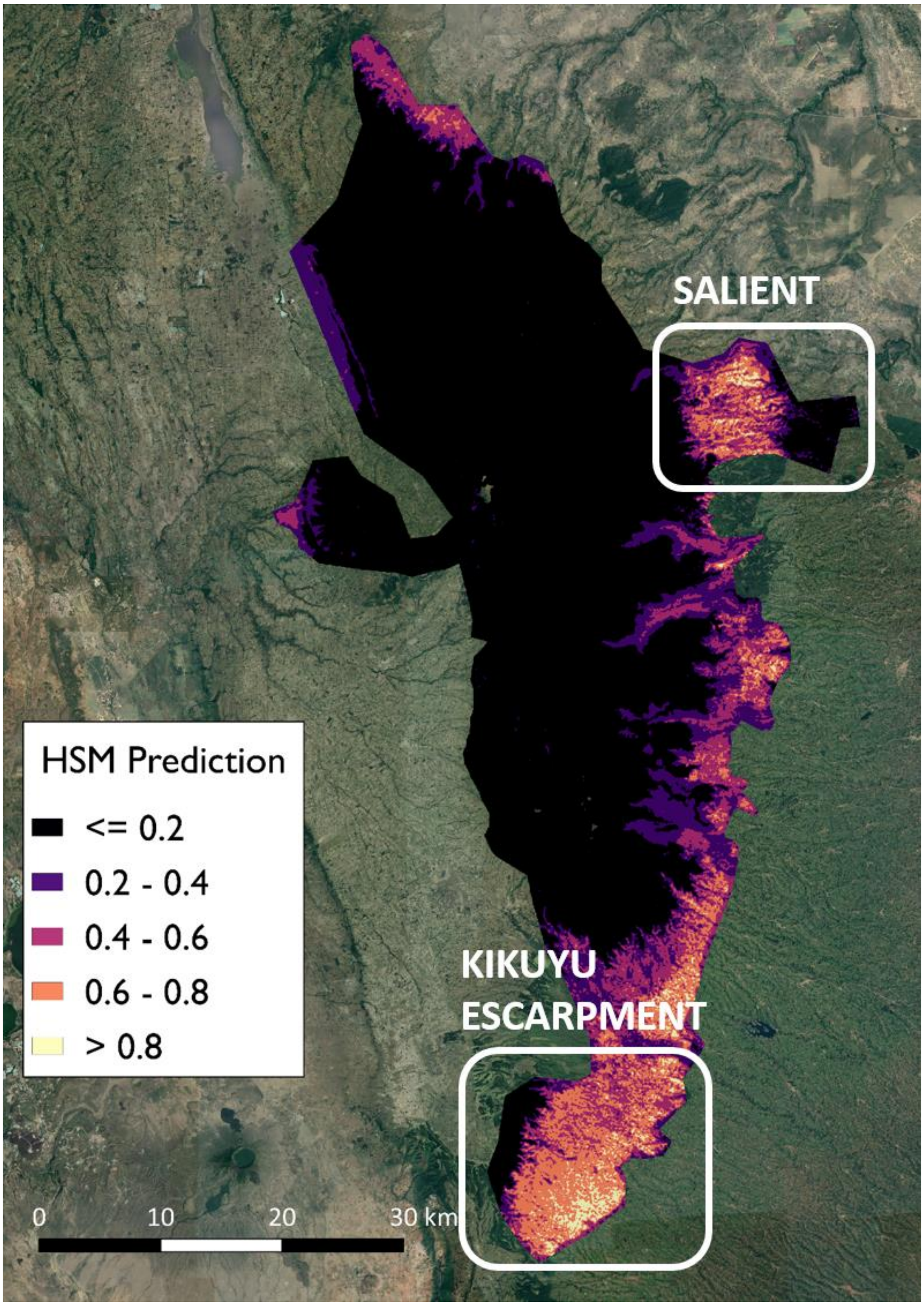

*Figure 1: Location of the Salient and Kikuyu Escarpment in the Aberdares.*

Two areas usually considered being the core component of the range, Mt. Kenya and Eburu, appear to be in a critical situation. In Eburu in particular, the male identified in this project, which was usually encountered in BSP footage, has not been captured throughout 2018 or in more recent BSP surveys. A more extensive camera trapping of Eburu conducted by BSP in 2018 failed to detect any bongo (BSP Reports), thus hinting at the possible extinction of bongo in the area. Results from Chapter 4 show how Eburu only partially consists of suitable habitat. The decline, and possible demise, of bongo in the area may be due to security issues such as poaching and land encroachment. However, there is a possibility that the population in Eburu was marginal compared to the other main areas which all show larger areas of suitable habitat. Therefore, I would suggest focusing conservation efforts in the other three areas where bongo are found.

The situation in Mt. Kenya is comparable to that of Eburu from a demographic perspective. A single female was usually, and still is, encountered in BSP surveys. However, this is atypical, as female bongo are highly social (Estes, 1992). Although females tend to leave the herd when calving, and hence would explain the sight of a solitary female in the area, no calf has ever been encountered since BSP started surveying the area in 2013. Results from Chapter 4 indicate a different situation from that encountered in Eburu though, as Mt. Kenya shows large areas of suitable habitat, also within the area currently surveyed by BSP (Figure 2). Therefore, more extensive camera trapping and monitoring should be undertaken to understand whether the population in the area is truly down to a single female. Nevertheless, due to the presence of habitat Mt. Kenya should be regarded as a possible location for future reintroductions.

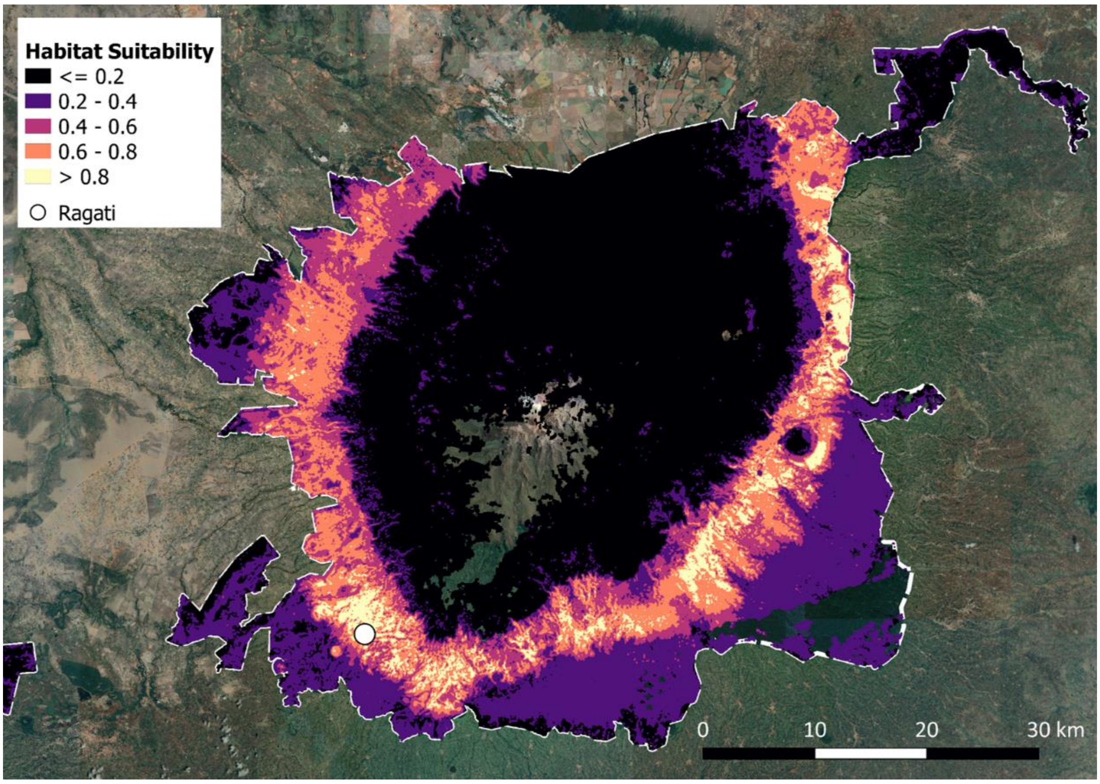

*Figure 2: Location of the area surveyed by BSP (Ragati) in Mt. Kenya.*

Some of the areas predicted by the species distribution model presented could become the focus of future monitoring, as hitherto never encountered bongo herds may inhabit these areas. One of the most interesting areas is the kikuyu escarpment (Figure 1). This area was found to comprise some suitable habitat, while the recent discovery of the only known golden cat population in Kenya (Hatfield et al., 2019) reinforces the idea that the area may be relevant for bongo conservation and Afromontane biodiversity at large.

However, the areas where suitable habitat may still be present are scattered throughout central Kenya. This fragmentation calls for actions aimed at increasing and facilitating the connectivity among present and future populations, as even if populations could be incremented in size thanks to demographic augmentations, fragmentation would inhibit vital connectivity and hamper the long-term viability of these wild populations (Frankham, 2010). The areas surrounding the mountains where bongo survive are highly anthropic, mainly consisting of agriculture (Figure 3). Therefore, the connection of these forest areas is difficult. Plans for the establishment of a wildlife corridor connecting the Aberdares and Mt. Kenya (Rhino Ark, 2017) would be essential to provide connectivity between an area with a healthy but small population, the Aberdares, and Mt. Kenya, which presents suitable habitat and could potentially support reintroduced populations. However, the scattered distribution of forest areas in Kenya means that bongo conservation may depend on including a form of assisted gene-flow (Frankham, 2010b; Aitken and Whitlock, 2013) in the species management. Individuals could be translocated between areas otherwise inaccessible with natural dispersion. Nonetheless, this aspect of bongo conservation will only become relevant once additional populations are established throughout Kenya montane forests.

### *Reintroduction and the Captive Population*

Restoring wild bongo populations implies reliance on the large captive population currently held in zoos and other facilities worldwide. Captive populations, in particular those held in zoos, play a major role in preserving a species (Conde et al., 2011). The bongo would be an ideal candidate to implement a management approach that aims at integrating captive and wild populations, the so-called One Plan Approach (Byers et al., 2013). The existence of a large captive population, combined with the presence of a captive breeding facility *in-situ*, MKWC, calls for greater integration of the wild and the captive populations and of their managers.

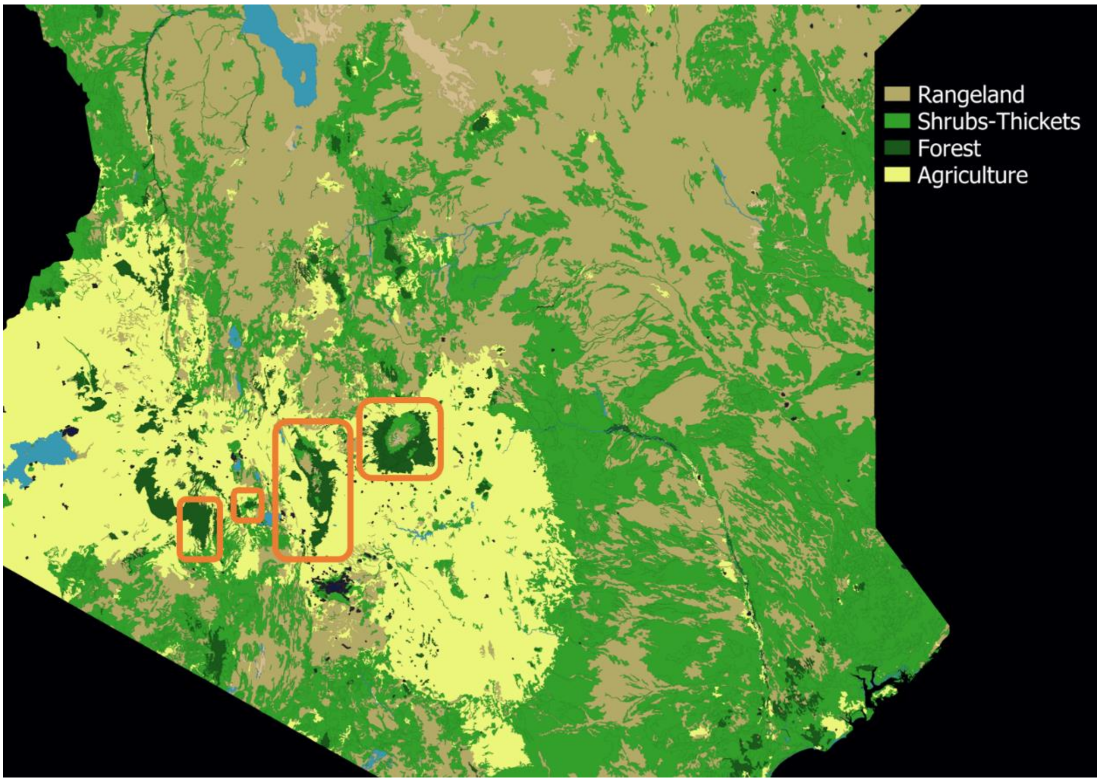

*Figure 3: Map showing land use in Kenya (data from: World Resource Institute). Areas inhabited by bongo are highlighted in orange.*

One of the main controversies in reintroduction biology, and bongo conservation is no exception, is the genetic makeup of the source population, due to the underlying risk of outbreeding depression (Waller, 2015). Evidence collected here, combined with existing literature on the subject (Faria et al., 2011; O'Donoghue et al., 2017; Svengren et al., 2017) indicate that this issue is not relevant for Mountain bongo. Although most of the evidence is restricted to mitochondrial DNA, the limited diversity found in the wild and in captivity should reassure managers that outbreeding is unlikely. Moreover, while males are usually to prefer over females in reintroduction due to the latent risk of mito-nuclear incompatibilities (Havird et al., 2016), results from Chapter 5 indicate that there is no reason to exclude females from future bongo reintroductions. Therefore, I see no impediment from a genetic point of view on the use of captive bred individuals for demographic augmentation of bongo in the wild.

The lack of genetic diversity unique to the captive population can be seen as a double edge sword. While outbreeding depression is unlikely, the zoo population may not provide what is needed for a genetic rescue of the wild populations (Whiteley et al., 2015). Genetic rescue relies on “fresh” genes to be imported into a dwindling or struggling population where inbreeding is limiting growth, and by augmenting the heterozygotes it would allow to overcome the effect of inbreeding (Bell et al., 2019). The lack of unique variation in captivity may indicate that little is to be gained, genetic wise, by following a genetic rescue approach. More insight into the differences at nuclear level between captive and wild individuals are needed before embracing this view, but currently available evidence in literature and presented here suggests this. Nevertheless, the captive population is of value to bongo conservation beyond its genetics. The low size of the only two functioning populations, Maasai Mau and Aberdares, calls for the use of reintroduction as an effort in boosting the number of individuals in the wild.

Therefore, survival of bongo depends on the captive population, and the effort to maximise representation of founders, and of lineages, must remain an imperative for the managers of this population. The assessment of matrilines showed that some lineages are highly unrepresented, and an immediate action that can be taken within the zoo community is to heighten representation of these. Additionally, the matrilines that were not assessed in this study should be examined. If additional variation is found in some of the non-sampled lineages then the case for using the captive population also for genetic rescue could become stronger. Moreover, a deeper and more detailed analysis of captive individuals relying on nuclear markers and genomic tools could better inform managers on whether certain lineages have managed to maintain more genomic variation, and these may be more valuable for reintroduction, as they could provide variation since lost in the wild. Therefore, implementing a one-plan approach within Kenya, by managing the captive population at MKWC concerted with management and protection of remnant wild populations could prove successful. MKWC could be a hub for the reintroduction of individuals in suitable areas. Besides, a continuous monitoring of the wild populations will swiftly inform managers on the need for actions targeted at these populations in case of lack of growth or decline.

## Conclusion

The project fulfilled its primary aim of providing information relevant for the conservation of Mountain bongo; however, it will prove successful only if the information here gathered and presented become integral to future conservation actions. The approach used in delivering this PhD project in collaborating with government authority (KWS) and with the local NGO at the frontline of bongo conservation (BSP) provides reasons for hope that the work here presented will fulfil its ultimate aim of helping the long-term conservation of this iconic antelope. The Mountain bongo could, and should, become a flagship for the threatened ecosystem it inhabits, that of the Afromontane forest of Eastern Africa and if by helping the bongo conservation the work here presented managed to also help conserve this astonishing ecosystem, this project would truly be a success.

## Future Research

### *Biodiversity Inventory of Maasai Mau and Southern Aberdares*

The relevance of Maasai Mau for bongo conservation prompts the need of a full assessment of whether this area could be important for other aspects of Afromontane biodiversity. Therefore, an inventorying project covering the area would not only elucidate on the species present in the area, but also help refining the area occupied by resident bongos. The prediction of suitable bongo habitat and the recent finding of golden cat (Hatfield et al., 2019) should heighten the interest in assessing the biodiversity of the Kikuyu Escarpment in the south of the Aberdare range. Both Maasai Mau and the Kikuyu

Escarpment are not fully protected, and expanding current knowledge on the assembly of species inhabiting them could precipitate conservation actions aimed at further protection of these areas.

### *Explore the Reasons for the Low Genetic Variation seen in Mountain Bongo*

The results from Chapter 5 are in agreement with previous research, but the limited scope of the analysis, confined to mtDNA control region, means that additional research implementing also nuclear markers or Next Generation Sequencing techniques is needed to further elucidate the relationship among the most important wild populations. Moreover, the low genetic diversity found in both wild and captive populations suggests that the variation was already limited at the time of the establishment of the breeding program, in the mid-20th century. An interesting study would consist in elucidating whether the current situation is due to a bottleneck event following the combination of habitat loss, hunting, and particularly rinderpest outbreaks; or if it relates to the central - marginal theory where populations at the margin of a species distribution show lower variation than those from core areas (Eckert et al., 2008). Assessing the demographic history of Mountain bongo relying on Museum samples and including samples from the nominal subspecies, the Lowland bongo, in other parts of the continent would help elucidate such questions.

### *Address the Challenges and Opportunities of Mountain Bongo Conservation in a Human Dominated Landscape*

Mountain bongo habitat is limited to highland areas of central Kenya. These are fragmented and isolated in a human dominated landscape. Therefore, while in Chapter 4 the influence of direct human disturbance was found to have negligible effects on bongo habitat selection, a deeper analysis of the role that anthropogenic factors may play in the long-term survival of this antelope is needed. Such information would refine the identification of optimal areas for reintroductions and it would inform managers on the need for protection in the bongo core areas. Nevertheless, such an analysis would also allow managers to have a better insight into the human dimension of bongo conservation, an aspect that is often overlooked in designing conservation actions (Salafsky et al., 2002).

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