GENETIC INSIGHTS INTO THE DYNAMICS OF HYBRIDIZATION BETWEEN AFRICAN ELEPHANT SPECIES

by
CLAIRE KATHERINE GOODFELLOW

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Student: Claire Katherine Goodfellow

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This dissertation has been accepted and approved in partial fulfillment of the requirements for the Doctor of Philosophy degree in the Department of Biology by:

William Cresko	Chairperson
Nelson Ting	Advisor
Caitlin Wells	Core Member
Peter Ralph	Core Member
Bart Johnson	Institutional Representative

and

Krista Chronister	Vice Provost for Graduate Studies

Original approval signatures are on file with the University of Oregon Division of Graduate Studies.

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DISSERTATION ABSTRACT

Claire Katherine Goodfellow

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Title: Genetic Insights into the Dynamics of Hybridization Between African Elephant Species

Current evidence strongly supports the existence of two African elephant species – forest (*Loxodonta cyclotis*) and savanna (*Loxodonta africana*) – which are both highly threatened due to poaching and habitat loss. However, conservation assessments and management decisions for these species are difficult at sites where they are known to overlap and hybridize due to a lack of detailed knowledge regarding species identity and population abundance. In this work, I survey the elephants at two protected areas of high conservation priority (Bwindi Impenetrable and Kibale National Parks) in Western Uganda, a region containing the world's largest African elephant hybrid zone. Using non-invasively collected genetic data, I elucidate the social and landscape-scale dynamics of these hybrid elephants for the first time.

In Chapter II, I develop a high-throughput amplicon sequencing approach within a fecal DNA-based Capture Mark Recapture framework to jointly infer the population sizes and species compositions of elephants living at the two study sites. I find contrasting patterns of population abundance and species distribution between the two parks and suggest that a mix of natural and human-related landscape-scale processes may influence the patterns of hybridization observed throughout Western Uganda today.

In Chapter III, I implement social network analysis to elucidate characteristics of a hybrid elephant social system for the first time. I find that social groups in the Western Ugandan

elephant population assort based on female philopatry rather than species identity and that group size correlates with genetic ancestry. I then sequence two uniparentally-inherited genetic markers and find that the history of hybridization in this region is context-dependent across both space and time.

Taken together, this body of work highlights the need for further research into the causes and consequences of hybridization between these two species, and it emphasizes that site-specific monitoring and management efforts will be critical to informing national and international conservation strategies for taxonomically undefined and admixed elephant populations across the African continent.

This dissertation includes unpublished co-authored material.

CURRICULUM VITAE

NAME OF AUTHOR: Claire Katherine Goodfellow

GRADUATE AND UNDERGRADUATE SCHOOLS ATTENDED:

University of Oregon, Eugene, OR
University of Notre Dame, Notre Dame, IN

DEGREES AWARDED:

Doctor of Philosophy, Biology, 2023, University of Oregon
Bachelor of Science, Biology, 2017, University of Notre Dame

AREAS OF SPECIAL INTEREST:

Molecular Ecology
Conservation Biology
Non-Invasive Genetic Monitoring

PROFESSIONAL EXPERIENCE:

Graduate Teaching Fellow, University of Oregon, 2017-2023

Science Literacy Program Fellow, University of Oregon, 2020

Teaching Assistant, UNDERC, University of Notre Dame, 2017

Research Assistant, Archie Lab, University of Notre Dame, 2015-2017

Research Assistant, Schafer Lab, University of Notre Dame, 2015

GRANTS, AWARDS, AND HONORS:

Graduate Research Forum Best Panel Presentation, University of Oregon, 2022

Donald E. Wimber Foundation Award, University of Oregon, 2021

Department of Biology Teaching Award, University of Oregon, 2020

NSF Graduate Research Fellowship Program Honorable Mention, 2019

Bernard J. Hank Family Endowment Fund Research Award, University of Notre Dame 2015, 2016

Braco Award for Excellence in Cell Biology Research, University of Notre Dame, 2015

PUBLICATIONS:

Goodfellow, C. K., Whitney, T., Christie, D. M., Sicotte, P., Wikberg, E. C., & Ting, N. (2019). Divergence in gut microbial communities mirrors a social group fission event in a black-and-white colobus monkey (*Colobus vellerosus*). *American Journal of Primatology*, **81**(10-11), e22966.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

The three living elephant species represent the last extant members of what was once a diverse and widely distributed order of over 160 ancient elephantids (Sukumar, 2003). Of these, the Asian elephant (*Elephas maximus*) ranges across India and Southeast Asia, while the forest elephant (*Loxodonta cyclotis*) and savanna elephant (*Loxodonta africana*) are endemic to the African continent (Sukumar, 2003). All extant elephants are considered to be both ecosystem engineers and keystone species, and they occupy important and functionally unique niches within their respective environments (Western, 1989; Poulsen et al., 2018). Notably, each of these species is also at risk of extinction in the wild, primarily due to poaching and habitat loss: both the Asian elephant and the African savanna elephant are presently classified as "Endangered" by the International Union for the Conservation of Nature (IUCN), while the African forest elephant is classified as "Critically Endangered" (Gobush et al., 2021a; Gobush et al., 2021b; Williams et al., 2020). Further complicating this threat of extinction, African forest and savanna elephants have been shown to hybridize in a small number of geographically restricted locations and can produce fertile offspring (Mondol et al., 2015; Kim & Wasser, 2019). However, to date, few studies have directly examined the dynamics of hybridization between these two species, nor evaluated the role that species-identity should play in national and international conservation and management efforts for Africa's elephants.

In this chapter, I provide a brief overview of the current understanding regarding species-level similarities and differences between African savanna and forest elephants. I then introduce the study site for this work - the largest known modern hybrid zone between these species - and provide an outline for this dissertation.

African Elephant Species – A Comparative Perspective

The taxonomic status of Africa's elephants has historically proved a matter of much debate. Although their ranges have drastically contracted in recent centuries, these animals were once widely distributed across much of sub-Saharan Africa (Rosencranz & Sehgal, 2017; Groves et al., 2000). The elephants which inhabit the savannas, grasslands, and woodlands across this broad distribution are commonly referred to as "savanna" or "bush" elephants (Blumenbach, 1797), while the smaller elephants which range throughout the tropical rainforests of Central and West Africa have been designated "forest elephants" (Matschie, 1900). Despite several compelling arguments for a full species status distinction between these animals (Allen, 1936; Frade, 1934; Barriel et al., 1999), the majority scientific consensus throughout the 20th century remained that there was a single African elephant species, *Loxodonta africana*, with two subspecies: *Loxodonta africana africana* in southern and East Africa and *Loxodonta africana cyclotis* in the Congo Basin and West Africa (Dudley et al., 1992; Laursen & Bekoff, 1978; Matschie, 1900; Barnes et al., 1996).

Concurrent evidence for an alternative, species-level classification for these animals came in two forms around the turn of the 21st century. First, Groves and Grubb (2000) published a study detailing consistent and quantifiable differences in cranial morphology between forest and savanna elephants and laid out an argument for a two species classification based on significant differences in morphology, ecology, and conservation status between the two elephant types (Grubb et al., 2000). The next year, Roca et al. (2001) published the first genetic study comparing Africa's elephants, which demonstrated that these animals exhibited high levels of fixed genetic variation at ancestry informative sites, consistent with a deep, species-level differentiation between lineages. These two findings, along with various follow up studies on

genomics, behavior, ecology, and life history, have since largely shifted the current scientific consensus in favor of two distinctive and deeply diverged African elephant species (Hart et al., 2021). However, since the publication of these studies, most conservation, government, and regulatory agencies have been hesitant to formally acknowledge two separate species due to geographically restricted hybridization between them (Kim & Wasser, 2019; Mondol et al., 2015). Below, I review the current state of knowledge about these two species and their hybrids and highlight areas for future study.

Morphology

Morphological characteristics constitute some of the best understood species-typical differences between Africa's two elephant species. Perhaps most notably, African savanna elephants are on average larger than forest elephants and have a different body shape profile (Grubb et al., 2000). Their shoulder height ranges from 2.2 - 4.0 meters, and they typically have a concave back shape; in contrast, forest elephants only stand 1.8 - 3.0 meters at shoulder height and are characterized by a straight to rounded back (Christy, 1924; Malbrant & Maclatchy, 1949; Morrison-Scott, 1947, Roeder, 1970; Groves et al., 2000). Both species exhibit sexual dimorphism, but this trait is more pronounced in savanna elephants (meters at shoulder height: female: 2.2 – 2.6, male: 3.2 – 4.0) than forest elephants (meters at shoulder height: female: 1.8 – 2.4; male: 2.4 – 3.0) (Groves et al., 2000). This difference between species has loosely been ascribed to more intense intrasexual competition between savanna elephant males (LaDue et al., 2021; Slotow et al. 2000; Hollister-Smith et al. 2007; Rasmussen et al. 2008; Roca, 2019), but no work to date has rigorously examined this claim. Toe nail number and ear shape have also been used to further distinguish these species: savanna elephants are thought to have five nails on their

front feet, four nails on their hind feet, and large, triangular ears; in contrast, forest elephants are thought to have only four nails on their front feet, three on their hind feet, and smaller, rounder ears (Sikes, 1971; Grubb et al., 2000). However, recent findings highlight variation in toe nail number within species for some African savanna elephant populations, suggesting that this trait may not be as useful as previously believed for taxonomic differentiation (Parker & Graham, 2017).

Differences in tusk appearance and composition between these species are directly related to their conservation, as the higher density tusks of forest elephants are especially targeted by poachers (AWF, 2023). Savanna elephant tusks are less dense, but are thicker, and curved to point outward, whereas forest elephants have thinner, straight tusks that point downward (Grubb et al., 2000). Finally, casual observation suggests that all savanna elephants have deep amber eyes, while forest elephants exhibit variation in eye color which can include amber, blue, green, and white (Turkalo & Fishlock, 2015). However, no studies to date have rigorously examined this claim.

Ecology

Africa's elephants have both been classified as keystone species due to the critical role that their foraging ecology and behavior play within their respective environments (Western, 1989; Poulsen et al., 2018). Savanna elephants are broadly characterized as browser-grazers, generally relying on grasses and forbs in the wet season and bushes and trees in the dry season (Cerling et al., 1999; Moss et al., 2001). These animals can have dramatic effects on plant diversity, and multiple studies have provided evidence for elephant-mediated habitat conversion from forest/woodlands to savanna/grasslands due to their propensity for selectively pushing over

large trees (Eltringham, 1982; Owen-Smith, 1988; Spinage, 1994). Although this destructive behavior likely contributed in part to the geographic patterns of open savanna-woodlands that we see across Africa today (Laws, 1970), it was deemed a problem for the management of protected areas in the mid 20th century, which in turn precipitated a trend of elephant culls to protect plant biodiversity (Caughley, 1976; Feeley, 1965; Pienaar et al., 1966; Astle, 1971; Whyte et al., 1998). These culls continued until the 1970s, when intense poaching began across East Africa and elephant population sizes sharply decreased (Blanc et al., 2007; Eltringham & Malpas, 1980). In addition to their distinctive foraging behaviors, members of this species also undergo long-distance seasonal migrations in response to water availability which have been linked to critical nutrient cycling and dispersal (Wall et al, 2013; Chase, 20017; Lindeque, 1995). As they migrate, savanna elephants' dung can homogenize the spatial heterogeneity of nutrients throughout the landscape, moving important minerals like potassium, sodium, magnesium, and calcium from nutrient rich sites to nutrient poor sites (Wolf et al., 2013).

Unlike their savanna-dwelling relatives, forest elephants are browser-frugivores, and play a unique role in the dispersal and recruitment of trees throughout the Congo Basin (Campos-Arceiz & Blake, 2011). These animals feed almost exclusively on the fruit and bark of tropical trees and will migrate over long distances to feed on seasonally available fruit (White, 1994). Sometimes considered the largest extant frugivore, this species disperses the seeds of over 300 different plants species (Campos-Arceiz & Blake, 2011), a subset of which are obligately elephant dispersed and do not recruit sufficient saplings without passing through an elephant's digestive tract (Beaune et al., 2013). Like savanna elephants, forest elephants have also been classified as ecosystem engineers: they maintain an extensive trail system throughout their ranges which is used by many other animals, including humans, to move through the dense

forests of the Congo Basin (Blake & Inkamba-Nkulu, 2004). They also pull down epiphytic plants (White et al., 1993) and push over trees (Wing & Buss, 1970; Omeja et al., 2014), the latter of which can both allow light into the dense forest's understory and facilitate increased carbon sequestration as aboveground biomass (Berzaghi et al., 2019).

Social Structure and Life History

The social structures and life histories of savanna elephants have been well characterized in several long-term studies where direct, individual-based observations have been collated over multiple decades (Moss et al., 2011; Wittemyer, 2001; Whitehouse & Hall-Martin, 2009). The elephant populations at two of these study sites in particular—Amboseli National Park (Moss et al., 2011) and Samburu National Reserve (Wittemyer, 2001), both in Kenya—have provided some of the most rigorous data underlying our current understanding of savanna elephants.

Overall, this species is characterized by male dispersal and female philopatry (Archie et al., 2007; Nyakaana & Arctander, 1999), and females exhibit a complex, four-tiered, fission-fusion social structure which is thought to be driven by seasonal shifts in resource availability (Wittemyer et al., 2005). A mother and her dependent offspring comprise the first tier of this structure ("family unit"), while the second tier ("core group") consists of several matrilineally related adult females, and their dependent offspring (Moss & Poole, 1983). Females generally range with these core groups year-round, and join additional core groups to associate in larger, third tier ("bond group") units during the wet season (Wittemyer et al., 2005). The fourth tier of savanna elephant sociality is called a "clan," which is defined as all of the bond groups which share a home range during the dry season and is considered to be a level of spatial rather than a social organization (Moss and Poole 1983; Moss 1988).

Home range size varies greatly in this species from 15 km^2 to up to 11,000 km^2 (Poole & Granli, 2009), but on average males — which disperse from their natal group upon reaching reproductive maturity (~10 years) — range over larger areas than do females (Wall et al., 2021; Moss & Poole, 1983). After dispersal, males will range either solitarily or in loose bachelor herds (Moss & Poole, 1983; Poole, 1982). Group size in savanna elephants is also highly variable across time: an average core group typically consists of 4-5 adult females and their dependent offspring, while herds of several hundred individuals can form during times of resource abundance (Moss & Poole, 1983; Archie et al., 2006; Poole & Moss, 2008). Although population specific demographic parameters vary, female savanna elephants broadly exhibit a median primiparous age of 12 - 14 years, and an average interbirth interval of 3 - 5 years (Wittemyer et al., 2013; Gough & Kerley, 2006; Moss, 2006). Reported generation times for savanna elephant populations have ranged from 17.4 - 24.1 years (Wittemyer et al., 2013; Moss, 2001), with an average population doubling time of ~20 years (Turkalo et al., 2017).

In contrast to the well-characterized demographic parameters and social structure of savanna elephants, less is known about the more elusive forest elephant. Both direct observation and indirect evidence point to smaller female group sizes in this species, typically consisting of a mother and her dependent offspring (Fishlock et al., 2008; Merz, 1986; Morgan & Lee, 2007; Munshi-South, 2011; Querouil et al., 1999; Schuttler et al., 2014b; Theuerkauf et al., 2000; White et al., 1993). These groups are also thought to be more disconnected than those in savanna elephants, and to exhibit a less complex social structure (Schuttler et al., 2014b; Fishlock & Lee, 2013; Hedwig et al., 2021). Due to the smaller group sizes characteristic of this species, both males and females disperse from their natal groups: males begin to disperse when they reach reproductive maturity (13-17 years), while females leave their natal group at first pregnancy (20-

23 years) (Fishlock et al., 2008; Turkalo et al., 2013, 2018). Furthermore, unlike in savanna elephants, it is thought that forest elephant males are mostly solitary and rarely range in bachelor herds (Turkalo et al., 2013).

Information about the life history traits of this species primarily derives from one long-term study: the Dzanga Forest Elephant Study at Dzanga Bai in the Central African Republic, which was initiated in 1990 and provides the only reliable individual-based demographic parameters for a forest elephant population (Turkalo, 2013). These limited data suggest that forest elephants exhibit delayed life histories relative to savanna elephants (Turkalo et al., 2017). The median primiparous age for the Dzanga Bai population is 23 years, with an average interbirth interval of 68 months for female elephants (Turkalo et al., 2017). Furthermore, the average generation time for this population is 31 years – one of the longest on record for any living species – and the population doubling time is up to three times longer than that of savanna elephants (Turkalo et al., 2018). Taken together, the slower life history of this species makes them particularly vulnerable to extinction (Turkalo et al., 2018; Poulsen et al., 2017).

Genetic Diversity and Evolutionary History

Africa's two elephant species diverged 2.6 to 5.6 million years ago (Palkapoulou et al., 2018) and are highly genetically differentiated (F_{ST}=0.643, Palkopoulou et al., 2018; F_{ST} =0.94, Roca et al., 2001; R_{ST}=0.90, Comstock et al., 2002). Forest elephants also exhibit consistently higher nuclear and mitochondrial genetic diversity than savanna elephants (Roca et al., 2001, 2005; Comstock et al., 2002; Rohland et al., 2010; Ishida et al., 2011; Palkopoulou et al. 2018). This difference has alternatively been attributed to 1) putatively decreased variance in reproductive success for forest elephant males relative to savanna elephant males and/or 2) a

population bottleneck in savanna elephants at the end of the Pleistocene (Roca, 2019). However, further work is needed to interrogate the causes and consequences of this species-level genetic difference.

Interspecies hybridization and introgression have recently been identified as surprisingly recurrent features of elephantid evolution (Palkopoulou et al., 2018), yet modern-day hybridization between elephant species is rare (Roca et al., 2001; Comstock et al., 2002) and no genomic signature of hybridization over the past ~1.3 million years has been detected in representatives of modern forest and savanna elephants (Palkopoulou et al., 2018). However, mitochondrial DNA derived from forest elephants has been detected in a number of putatively pure savanna elephant populations across East and southern Africa (Debruyne, 2005; Ishida et al., 2011, 2013; Roca et al., 2005, 2007), indicating that ancient hybridization and introgression did at one time occur between these species.

Conservation Status

Both African elephant species face severe rates of decline due to poaching and habitat loss (Chase et al., 2016). However, forest elephants face a more immediate threat of extinction than savanna elephants (Gobush et al., 2021b). Savanna elephants are currently classified as "Endangered" by the IUCN, with fewer than 400,000 individuals remaining in the wild following a 60% decline over the past three generations (Gobush et al., 2021a). In contrast, forest elephants are currently classified as "Critically Endangered," as there are only ~56,000 individuals remaining following an 86% decline over the past three generations (CITES, 2022; Gobush et al., 2021b). It is thought that the harder tusks of this species make their ivory more desired by poachers than the less dense ivory of savanna elephants (AWF, 2023). Furthermore,

the slower life histories of forest elephants relative to savanna elephants indicate that poached populations could take up to three times longer than savanna elephant populations to recover (Turkalo et al., 2018; Poulsen et al., 2017). These threats are notably exacerbated by the habitats in which forest elephants are found: the dense forests which these elephants occupy make traditional sight-based census methods unfeasible (Barnes & Jensen, 1987; Burnham et al., 1980), and drastically limit the data which can be generated about the population trends, basic biology, and management needs of this species (Fishlock & Breuer, 2015).

Limitations and Open Questions

The species-typical differences between African forest and savanna elephants detailed throughout this section primarily derive from a small number of well-characterized elephant populations at long-term study sites (Moss et al., 2011; Wittemyer, 2001; Turkalo et al., 2013). Although data like these are critically important to our understanding of these species, they also reflect a well-documented geographic bias within elephant research, wherein publications are not representative of the distribution of elephants across the African continent (Gross & Heinsohn, 2023). For example, the most recent continent-wide elephant census revealed that South Africa and Kenya together harbor only ~10% of the remaining savanna elephants (Chase et al., 2016), but constitute ~49% of the published research for this species (Gross & Heinsohn, 2023). Because each of these over-represented study sites encompasses only one African elephant species, it remains unclear the degree to which the "species-typical" traits detailed above are truly species typical, and it is possible that some of these traits might in fact be population specific and could exhibit plasticity in different environmental contexts.

Western Uganda - A Modern Day Elephant Hybrid Zone

Hybrid zones between species present an important opportunity for insight into questions such as this, as they are a "natural laboratory" in which animals with different genetic ancestry are subject to similar ecological constraints in a common environment (Barton & Hewitt, 1985; Hewitt, 1988). African forest and savanna elephants exist in sympatry around the Congo Basin, but have only been demonstrated to hybridize at a small subset of these locations (Mondol et al., 2015; Kim & Wasser, 2019). Of all the hybrids which have been detected across the continent, 62.6% have occurred in a single location along the border of Uganda and the Democratic Republic of the Congo (DRC) in East Africa's Albertine Rift, the region which is considered to be the primary hybrid zone between these two species (Kim & Wasser, 2019).

This region represents a natural ecotone between the rainforests of the Congo Basin and the dry savanna woodlands of East Africa and encompasses a mosaic of habitat types including montane and submontane forests, savanna woodlands, and grasslands (Plumptre et al., 2007). The elephant population inhabiting this landscape is also characterized by a unique history of poaching-induced decline linked to the political history of the region (Mondol et al., 2015; Bonnald et al., 2021). The political upheaval and military occupancy of Idi Amin's violent dictatorship (1971-79) led to rampant poaching of elephants living in Uganda (Aleper & Moe, 2006; Keigwin et al., 2016). It is thought that those elephants in Uganda which were not poached during this time fled west across the border to the DRC, where they remained until the 1990s (Keigwin et al., 2016). When civil war (and, subsequently, increased poaching) began in the DRC at this time, it is hypothesized that surviving elephants again fled across the border, returning to Uganda (Keigwin et al., 2016).

Several alternative hypotheses have been proposed to explain the unique elephant hybrid zone found in this region. The most frequently cited hypothesis is that this hybrid zone is human-induced, and that the elevated levels of hybridization detected in this region are the result of the distinctive patterns of poaching and transboundary migration described above (Mondol et al., 2015; Bonnald et al., 2021). In this scenario, many of the elephants fleeing Uganda to the DRC would have been savanna elephants pushed outside of their typical ranges and into the ranges of forest elephants in the DRC. Then, in the 1990s, both savanna and forest elephants would have fled the DRC back to Uganda.

Alternatively, it has also been hypothesized that this hybrid zone could be more ancient (Mondol et al., 2015). The presence of forest elephant derived mitochondrial DNA in pure savanna elephant populations throughout East and southern Africa suggests that shifting ecotones over a longer, more ancient timeframe may have resulted in range contractions and expansions for these two species, with limited hybridization and introgression occurring where the ranges meet (Debruyne, 2005; Eggert et al., 2002; Ishida et al., 2011, 2013; Johnson et al., 2007; Nyakaana et al., 2002). In this scenario, the hybrid zone observed in Western Uganda today would be considered natural, and simply a modern-day window into elevated levels of hybridization which have followed a shifting forest-savanna transition zone through time (Oliveras & Malhi, 2016).

The two hypotheses detailed above are not mutually exclusive, and it is also possible that other factors like land conversion might contribute to the elevated levels of hybridization that we observe in Western Uganda (Mondol et al., 2015). Further work is needed to examine the dynamics and history of hybridization throughout this region.

Dissertation Outline

The overarching objective of this dissertation is to elucidate the spatial, historical, and social dynamics of two sites embedded in the largest known modern hybrid zone between forest and savanna elephants. Bwindi Impenetrable and Kibale National Parks are two protected areas in Western Uganda for which species-level elephant population data are limited due to the dense forests that characterize each of these sites. Throughout this dissertation, I examine the fine-scale dynamics of the elephant populations at these two sites, and ultimately seek to inform on the consequences of hybridization and the role that species identity should play in the conservation and management of Uganda's elephants. Specifically, my work addresses the following questions:

1) How are hybrids distributed across the landscape in Western Uganda, and how many of each elephant type (i.e., savanna, forest, hybrid) occur throughout this region?

2) What are the social dynamics of a hybrid elephant population, and how does species identity intersect with various aspects of social structure and organization?

3) What is the history of hybridization between savanna and forest elephants in Bwindi Impenetrable and Kibale National Parks, and is there evidence for sex-biased hybridization?

In Chapter II, I integrate high-throughput amplicon sequencing (HTAS) within a fecal DNA-based Capture Mark Recapture (CMR) framework to jointly infer the population sizes and species compositions of elephants living in Bwindi Impenetrable and Kibale National Parks. This chapter is titled "Elephants inhabiting a hybrid zone in Western Uganda exhibit site-specific patterns of abundance and species identity." In addition to myself, this work is co-authored by Nelson Ting, Daniella E. Chusyd, Caitlin P. Wells, Dennis Babaasa, Patrick A. Omeja, Charles

Tumwesigye, Michael D. Wasserman, Colin A. Chapman, Richard Mutegeki, and Jena R. Hickey. This chapter provides baseline data for the Uganda Wildlife Authority's conservation management of the elephants living in Bwindi Impenetrable and Kibale National Parks in Western Uganda, contributes valuable data to continent-wide elephant inventories for forest and savanna elephants, and serves as a launching point for ongoing study and monitoring of the elephants in these two densely forested parks.

In Chapter III, I then characterize the social system of a hybrid elephant population for the first time. This work is titled "Patterns of mixed-species association and hybridization in the elephants of Bwindi Impenetrable and Kibale National Parks, Uganda," and is co-authored by myself, Nelson Ting, Daniella E. Chusyd, Caitlin P. Wells, Dennis Babaasa, Patrick A. Omeja, Charles Tumwesigye, Michael D. Wasserman, Colin A. Chapman, Richard Mutegeki, and Jena R. Hickey. Although behavioral differences between African forest and savanna elephants have been documented in isolation, no work to date has examined the social dynamics of mixed-species elephant populations or the role that genetic ancestry plays in maintaining species-typical behavioral phenotypes. Because these species are characterized by distinct social systems, hybrid populations representing individuals with a range of genetic ancestries offer a unique opportunity for insight into factors underlying species-typical behaviors in a common environment. In this chapter, I implement social network analysis and sequence uniparentally-inherited genetic markers to elucidate various aspects of the social system of a hybrid elephant population for the first time.

Finally, Chapter IV summarizes my results, integrates my findings, and discusses implications of my work for the conservation and management of Africa's elephants.

CHAPTER II

ELEPHANTS INHABITING A HYBRID ZONE IN WESTERN UGANDA EXHIBIT SITE-SPECIFIC PATTERNS OF ABUNDANCE AND SPECIES IDENTITY

Contributions

This chapter is co-authored by Claire K. Goodfellow, Nelson Ting, Daniella E. Chusyd, Caitlin P. Wells, Dennis Babaasa, Patrick A. Omeja, Charles Tumwesigye, Michael D. Wasserman, Colin A. Chapman, Richard Mutegeki, and Jena R. Hickey. Claire K. Goodfellow, Daniella E. Chusyd, Jena R. Hickey, Richard Mutegeki, and Michael D. Wasserman coordinated sample collection efforts. Claire K. Goodfellow contributed to sample collection, and was responsible for all genetic lab work, data analyses, and the writing of this manuscript. Claire K. Goodfellow, Nelson Ting, Daniella E. Chusyd, and Caitlin P. Wells contributed to study design. Patrick A. Omeja, Dennis Babaasa, and Colin A. Chapman provided guidance and intellectual support.

Introduction

Recent advances in sequencing technology have revealed a surprisingly high degree of both ancient and ongoing hybridization across a wide range of taxa (Payseur & Rieseberg, 2016; Taylor & Larson, 2019). This gene flow between species can exert critical influence on the adaptation, evolution and survival of species, and can be both a natural evolutionary process (Arnold, 1992; Gompert et al., 2017) and a consequence of anthropogenic activity (Allendorf et al., 2001; Grabenstein & Taylor, 2018). From a population management perspective, the increased ability to detect these hybrids using genetic tools demands the use of increasingly complex decision-making frameworks by wildlife authorities and practitioners (Allendorf et al.,

2001; Fitzpatrick et al., 2015; Jackiw et al., 2015; Quilodrán et al., 2020) given that small populations of rare or endangered species are especially prone to genetic swamping from hybridization with closely related domestic (Gottelli et al., 1994; Oliveira et al., 2008; Wells et al., 2019), introduced (Vuillaume et al., 2015; Riley et al., 2003), or more abundant sympatric species (Gese et al., 2015; Souza Arantes et al., 2020; Lima et al., 2019). Additionally, because many regulations are designated at the species level and do not recognize the legal protection of hybrids, hybridization can compromise protections for the hybrid offspring of endangered species (Allendorf et al., 2001; Draper et al., 2021).

Conservation authorities face this particular challenge when designating protections for Africa's elephants, which are declining across the continent due to poaching, human-wildlife conflict and habitat loss (Chase et al., 2016; Poulsen et al., 2017). Both morphological (Grubb et al., 2000) and genetic (Roca et al., 2001; Palkopoulou et al., 2018) data support the recognition of two African elephant species: the forest (*Loxodonta cyclotis*) and savanna elephant (*Loxodonta africana*). These animals constitute two distinct lineages which diverged between 2.6 and 5.6 million years ago (Palkopoulou et al., 2018), and which differ in distribution (Kim & Wasser, 2019; Mondol et al., 2015; Roca et al., 2001), behavior (Archie & Chiyo, 2012; Athira & Vidya, 2021; Schuttler et al., 2014b) and life history traits (Turkalo et al., 2018). However, due to geographically restricted hybridization between them (Kim & Wasser, 2019; Mondol et al., 2015), most conservation, government and regulatory agencies have been hesitant to formally acknowledge two separate species. This is because "lumping" African elephants into one conservation unit simplifies both international law enforcement efforts and local management decisions and prioritizes full protection for hybrids (CITES, 2022). However, it also artificially

inflates population numbers, which can obscure species-level trends and lead to negative conservation outcomes (Morrison et al., 2009).

In 2021, the International Union for the Conservation of Nature's African Elephant Specialist Group (IUCN AfESG) officially altered its position on this topic, splitting its listing status for Africa's elephants which were previously listed together as "Vulnerable" (Hart et al., 2021). This change required separate population assessments for the two species and resulted in a new "Endangered" listing status for savanna elephants (Gobush et al., 2021a), which have declined by 60% over the past three generations, and a "Critically Endangered" status for forest elephants, which have declined by more than 80% over the past three generations (Gobush et al., 2021b; CITES, 2022). This decision highlights the different threats of extinction faced by the two species, but also poses a series of new challenges (Hart et al., 2021), including a crucial need to generate high-resolution, species-level distribution data for taxonomically undefined priority sites across the African continent (Bauer et al., 2021).

One area with various priority sites is Western Uganda, a region which harbors the largest known modern hybrid zone between African forest and savanna elephants (Kim & Wasser, 2019; Mondol et al., 2015). While elephants ranged freely throughout this region as recently as 1929 (Brooks & Buss, 1962), they are now largely confined to a network of protected areas embedded in an agricultural matrix (UWA, 2016). Notably, the dense vegetation and steep topography of several of these protected areas have made traditional sight-based census methods unfeasible, and reliable census counts and species distribution data are lacking for elephant populations living in these parks (Plumptre et al., 2008). The most widely used method to survey elephants in forested areas like these is the line transect distance sampling (LTDS) method, which relies on both accurate dung counts along a series of replicated transects and seasonally

accurate defecation and dung decay rates for every surveyed site (Barnes & Jensen, 1987; Burnham et al., 1980). Importantly, although this method is well established and widely used, it does not provide species-level population data, making it less useful for species identification in taxonomically mixed or undefined elephant populations. One alternative to this method which enables joint inference of both census population size and species distribution in taxonomically undefined populations is a fecal DNA-based Capture Mark Recapture (CMR) approach (Brand et al., 2020; Eggert et al., 2003; Gray et al., 2014; Hedges et al., 2013, Laguardia et al., 2021b). Like the LTDS method, this approach relies solely on elephant dung. However, it does not require previous knowledge about site-specific parameters such as defecation and dung decay rate to accurately infer population size, and thus shows promise as a method for jointly generating species identity and abundance data at understudied sites.

Here, we implement a fecal DNA-based CMR approach to update population estimates for elephants in two forested sites in Western Uganda: Bwindi Impenetrable National Park and Kibale National Park. Specifically, we apply a new high-throughput amplicon sequencing protocol to jointly infer the population sizes and species compositions (ie: forest, savanna, hybrid) of elephants in these data-deficient sites. We then directly compare our results to a comparable, recent LTDS census in Kibale National Park to assess the feasibility of this method for directly informing the Uganda Wildlife Authority's species-level conservation management plan for elephants.

Methods

Study Area

Bwindi Impenetrable and Kibale National Parks are two forested sites which are part of

a greater, transboundary network of 11 protected areas located along the borders of Uganda, Rwanda, and the Democratic Republic of the Congo (DRC) (Figure 1). Together, these parks make up the Greater Virunga Landscape (GVL), a globally important site for its high rates of endemism and biodiversity (Plumptre et al., 2007). Kibale National Park is located approximately 140 km north of Bwindi Impenetrable National Park, and although these parks were historically connected via the GVL, it is thought that Bwindi's connection to this larger landscape was fully severed by land conversion outside of its boundaries by the late 1950s (Brooks & Buss, 1962; Plumptre et al., 2007). Annually, the GVL experiences wet seasons from March – May and September – November, and dry seasons from December – February and June – August (Plumptre et al., 2008).

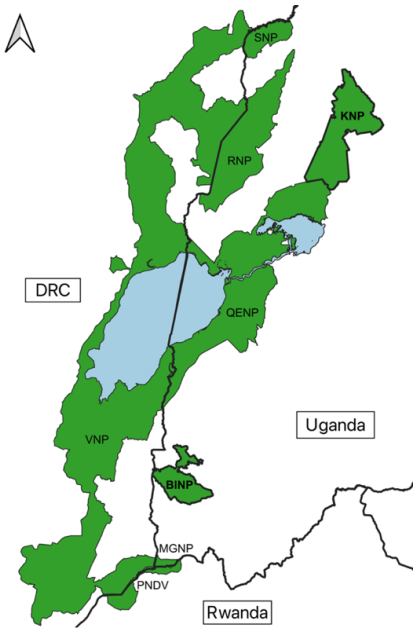


Figure 1. Map showing the protected areas which together make up the Greater Virunga Landscape (GVL). This network spans the borders of Uganda, Rwanda, and the DRC and includes both Kibale National Park (KNP) and Bwindi Impenetrable National Park (BINP), two forested protected areas located in Western Uganda. Abbreviations for the parks above are: KNP - Kibale National Park; BINP - Bwindi Impenetrable National Park; MGNP - Mgahinga Gorilla National Park; RNP - Rwenzori National Park; VNP - Virunga National Park; PNDV - Parc National des Volcans; SNP - Semuliki National Park; QENP - Queen Elizabeth National Park. (Map generated in QGIS v.3.30. Data Source: National Forest Authority, 2007).

Kibale National Park (KNP) is a 795 km^2 protected area located near the foothills of the Rwenzori mountains in Western Uganda (0°13'-0°41'N and 30°19'- 30°32' E). The altitudinal range for this park varies from 1,110 meters to 1,590 meters above sea level, and its vegetation is broadly classified as medium-altitude semi-deciduous forest, medium-altitude moist evergreen forest and grasslands (Chapman & Lambert, 2000; Langdale-Brown et al., 1964). The park is primarily situated within an agricultural matrix, with the exception of a largely degraded corridor which was established in 1926 to facilitate elephant movement between KNP and Queen Elizabeth National Park to the south (Chapman & Lambert, 2000; UWA, 2016); historically, elephants undertook seasonal migrations between these parks (Wing & Buss, 1970), but the degree to which this corridor is used today remains unknown (Plumptre et al., 2008). Elephant population estimates for this park have included 600 in 1961 (Wing & Buss, 1970), 262 in 2001 (UWA, 2016), 393 in 2005 (Wanyama et al., 2010), 487 in 2010 (UWA, 2016), and 566 in 2019 (Aleper et al., 2021). A recent study conducted in the Sebatoli region, a 25 km^2 area at the northernmost part of KNP, found primarily hybrid (81.3%) and savanna elephants (18.7%) in KNP (Bonnald et al., 2021). However, this region constitutes only ~3% of the park's total area and is characterized primarily by regenerating forest, which is not representative of the park's vegetation as a whole (Chapman & Lambert, 2000; Langdale-Brown et al., 1964). Thus, it remains unknown whether these results are representative of elephant species prevalence throughout KNP.

Bwindi Impenetrable National Park (BNP) is a 331 km^2 protected area located in the Rukiga highlands of southwestern Uganda (0°53' to 1°08' S; 29°35' to 29°50' E). The park varies in altitude from 1,160 meters to 2,600 meters above sea level, and its rugged terrain is characterized by a series of steep-sided ridges separated by narrow, poorly-drained valleys

(Butynski & Kalina, 1993). The park is managed as two "sectors"—the "Northern" and "Southern" sectors–which are connected by a 1.5 km wide forested corridor. The vegetation is broadly classified as medium-altitude moist evergreen forest and high-altitude submontane forest (Langdale-Brown et al., 1964). Although elephants historically ranged throughout the entire park (Butynski, 1986), a survey conducted from 1992-1993 found that the park's elephant population at the time limited its range to only 186 km^2 of the Southern Sector, putatively due to a recent history of intense poaching pressure in the park (Babaasa, 1994). Recent estimates for this park's elephant population have included 30 (Butynski, 1986), 22 (Babaasa, 1994), 30-50 (Plumptre et al., 2008), and 43 (UWA, 2016), though the last comprehensive census for BINP's elephants was conducted in 1993. Limited data suggest that both forest elephants and hybrids are found in this park (Mondol et al., 2015), though no high resolution park-wide genetic survey has previously been conducted.

Sampling Scheme

Dung samples were non-invasively collected from across KNP from November 2020 to August 2021, except in February 2021 due to changes in standard operating procedures associated with COVID-19. Sample collection occurred for 6 to 7 days consecutively, every other week. During this time, the field team (3-5 individuals) would set up a base camp out of Uganda Wildlife Authority (UWA) outposts, or when not available, would establish base camps in the forest. After two weeks, the team would rotate to a different basecamp in a different area of the park. The team used informants, crop raiding events, known elephant hotspots, and elephant tracks to search for dung. For each fresh sample, the outside of a minimum of two dung

boli were swabbed using an Isohelix Buccal swab, and each swab was placed into a vial containing 1.5 mL of RNAlater (hereafter referred to as “swab samples”).

In BINP, sample collection was implemented over two sampling bouts. The first bout was conducted by the Greater Virunga Transboundary Collaboration from October – December, 2018 as part of a park-wide sweep survey for mountain gorillas and other large mammals (Hickey et al., 2019). Briefly, two field teams (4-5 individuals each) simultaneously swept 40 pre-determined units of the park in 14-day shifts by walking reconnaissance routes spaced ~500 meters apart in each unit. When fresh elephant dung piles judged to be <48 hours old were encountered by teams during this census, approximately 15 mL of elephant dung were georeferenced and placed into 15 mL of RNAlater (hereafter referred to as “pinch samples”). The second sampling bout was conducted by one field team and took place July – September 2019. Sample collection occurred for 5 to 6 days consecutively, every week. After each week, the team would rotate to a different basecamp in a different area of the park. Prior to the survey, a grid of 2x2 km cells was overlaid over a map of the park, and cells containing potential “hotspots” were identified based on vegetation (fruiting trees, bamboo), terrain type (swamps, valleys, water sources) and ranger knowledge of the area. On each survey day, 1-2 non-adjacent hotspot cells were visited and searched by a field team of 2-3 individuals. Cells were not re-visited. Upon entering a cell, elephant trails were followed, and in the case of a newer elephant trail being encountered that trail was followed. Dung piles judged to be <48 hours old were georeferenced and swab samples were taken, with a sample being taken from each unique pile in the case of associated piles. All samples were stored in a cool, dry area in the field for up to 6 weeks before being transferred to a −20°C freezer. After shipment to the University of Oregon, samples were again stored at −20°C until DNA extraction.

Genetic Analysis

DNA extraction and genotyping were conducted in the Ting lab at the University of Oregon. Pinch samples were extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen), with modifications to the manufacturer protocol (Archie et al., 2003). Briefly, these modifications were: 1) 500 uL of fecal slurry was initially digested for 10-12 hours in 1 mL buffer ASL and 1 mg Proteinase K in a 55°C incubator, shaking at 300 rpm; 2) an increased total volume of 1,800 - 3,000 uL was centrifuged through each spin column; and 3) prior to the final elution, 75 uL of buffer AE was incubated on the spin column at room temperature for 30 minutes. To extract DNA from swab samples, the swab head was transferred along with 500 uL of RNAlater from its collection vial into buffer ASL and Proteinase K for overnight incubation as described above. Following overnight incubation, the swab head was removed from the tube, and extraction proceeded as described for pinch samples.

Each DNA extract was sexed according to the protocol in Ahlering et al. (2011) and was genotyped at 14 microsatellite loci which have previously been shown to be polymorphic in African elephants. These loci were: FH71 (Comstock et al., 2000), LA5, LA6 (Eggert et al., 2000), LafMS03, LafMS04 (Nyakaana et al., 2005), and Lcy-M8, Lcy-M16, Lcy-M17, Lcy-M26, Lcy-M27, Lcy-M29, Lcy-M30, Lcy-M40, and Lcy-M44 (Gugala et al., 2016). Genotyping was conducted using a two-step, high-throughput amplicon sequencing (HTAS) approach (Barbian et al., 2018). Primers for microsatellites were ordered with an Illumina sequencing tag attached to the 5' end of both forward and reverse primers (FW primer overhang: 5′ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG 3′; RV primer overhang: 5′ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG 3′). The 14 loci were then amplified in 2 pooled reactions per sample (Pool 1: LafMS03, LafMS04, Lcy-M8, Lcy-M26, Lcy-M27,

Lcy-M29, Lcy-M44; Pool 2: LA5, LA6, FH71, Lcy-M16, Lcy-M17, Lcy-M30, Lcy-M40). DNA was amplified in 15 uL reactions consisting of 7.5 uL Qiagen Multiplex Mastermix, 4.33 uL H_2O, 1.2 uL 10X primer pool, and 2 uL DNA. PCRs were performed in a Nexus Gradient thermocycler. Amplification conditions consisted of an initial denaturation step at 95°C for 15 minutes, followed by 45 cycles of 94°C for 30 seconds, 58°C for 90 seconds, and 72°C for 90 seconds. This was followed by a final extension step at 72°C for 10 minutes. After amplification, 5 uL of each of the two pools was combined by sample into 10 uL reactions, subjected to a 1.8X cleanup following the MagBind RxNPure Plus manufacturer's protocol, and diluted 1:100 into H_2O.

A second PCR was then performed to ligate dual-indexed adapters to the amplicons. This barcoding PCR was performed in 10 uL volumes consisting of 4 uL Qiagen Multiplex Mastermix, 0.5 uM i5 and i7 adaptors (Nextera XT Index Kit v2; Illumina), and 5 uL diluted amplicon pool (Lepais et al., 2020). Amplification conditions were: 95°C for 5 minutes followed by 10 cycles of 95°C for 30 seconds, 59°C for 90 seconds, and 72°C for 30 seconds. This was followed by a final extension step at 68°C for 10 minutes. Barcoded libraries were cleaned up for a second time at 1.8X following the MagBind RxNPure Plus manufacturer's protocol and quantified using the Quant-IT dsDNA HS kit (Thermofisher). 384 unique dual-indexed libraries were pooled equimolarly per run and sequenced on an Illumina MiSeq at the University of Oregon Genomics & Cell Characterization Core Facility (GC3F) using v3 chemistry at 375 forward read cycles (Barbian et al., 2018).

Population Genetic Analyses

Reads were demultiplexed by sample and adapters were trimmed and filtered for quality (Q>20) using cutadapt v. 3.5 (Martin, 2011). Samples were demultiplexed by locus and genotype calls were implemented using the program CHIIMP (Barbian et al., 2018). We used a length buffer of 100 bp for locus demultiplexing, a minimum read depth of 150 reads per locus (Salado et al., 2021) and 10 reads per allele for genotype calling. To validate genotype calls after CHIIMP analysis, we visually inspected histograms of read lengths and manually called genotypes using a custom python script. We required that consensus genotypes be confirmed in two or more replicates for heterozygous calls, and in three replicates for homozygous calls. We used GENEPOP v. 4.7.5 to test whether any loci exhibited Linkage Disequilibrium (LD). GenAlEx v. 6.51b2 was used to generate standard metrics of genetic diversity (Ho, He, F_{ST}), to test for deviations from Hardy Weinberg Equilibrium (HWE), and to identify unique individuals. P(ID) and $P(ID)_{sib}$ were calculated for our panel of 14 loci using GenAlEx.

Census Population Size Estimates

We used the R package capwire v. 1.3 to implement CMR analyses for each park. The statistical framework implemented in this package is optimized for use with non-invasive genetic samples and generates population size point estimates based on capture class frequency data (Pennell et al., 2013). Under our sampling design, samples with identical multilocus genotypes which were collected from the same location within the same 30-day sampling bout were considered "false recaptures," and were excluded from further analyses. We then fit two different models to our data: the Equal Capture Model (ECM) and the Two-Innate Rates Model (TIRM). The ECM assumes that all individuals in a population have an equal probability of being

detected, while the TIRM assumes two different classes of individuals which vary by capture probability. For each model, we generated point estimates for population size for each of the two parks using a maximum population size of 3,000 individuals. We then implemented parametric bootstrapping (N=100) to generate 95% confidence intervals around each point estimate. A likelihood ratio test (N=100 bootstraps) was used to determine which of the two models better fit the data for each park.

Species Assignment

A set of 19 putatively pure samples were genotyped and analyzed alongside the samples from KNP and BINP to serve as positive controls for species assignment analyses (*L. cyclotis*, n=9; *L. africana*, n=10; Table S1) Bayesian clustering analysis (K=1 through 5) was implemented in STRUCTURE v. 2.3.4 (Pritchard et al., 2000) using the Admixture model. Analyses were run at two different scales: 1) for each park separately; and 2) for the two parks combined. At each scale, we also implemented analyses with and without the LOCPRIOR parameter set to TRUE. For all analyses, the parameters INFERALPHA and POPALPHAS were set to TRUE. Five MCMC replicates were run per K, each with an initial burn-in of 200,000 followed by 4,000,000 iterations.

Following STRUCTURE analyses, we identified the K with the highest support in this population using STRUCTURE HARVESTER v. 0.6.94 (Earl & vonHoldt, 2012), and classified individuals as pure forest, pure savanna, or hybrid using two different methods. For the first method, we implemented a standardized cutoff to classify individuals based on STRUCTURE output. Using this method, individuals which showed a posterior ancestry proportion (Q) greater than or equal to 0.9 for either of the two putative populations (K=2) was classified as a “pure”

species for that cluster. This cutoff has been demonstrated to provide an optimal threshold for distinguishing between hybrid and parental types when using a small number of molecular markers (Vähä & Primmer, 2006) and has been widely used across a diverse range of taxa (e.g. Bohling & Waits 2011; Latch et al., 2011; Beaumont et al., 2001; Barilani et al., 2007; Trigo et al., 2008; Sanz et al., 2009). Individuals showing Q values < 0.9 for either ancestry-specific cluster were considered to be admixed, and classified as hybrid. For our second classification method, we used the R package EBHybrids v. 0.991 (Mondol et al., 2015) to classify individuals by species and to assign each sample a hybrid probability. This package uses an empirical Bayes method to quantify the likelihood of a sample being from a pure savanna elephant, a pure forest elephant or a hybrid based on STRUCTURE results, locus-specific allelic dropout rates, and a reference panel of putatively un-admixed individuals. As has been previously done (Bonnald et al., 2021), each individual was classified into a species category at three different likelihood thresholds (50%, 80%, and 95%). At 95% stringency, there is strong confidence that no pure forest or savanna elephants are being classified as hybrids; alternatively, at 50% stringency, there is greater confidence that no hybrids are being classified as pure species.

Results

Sample Collection and Individual Identification

A total of 399 samples from Western Uganda (KNP, n=256; BINP, n=143) were collected and analyzed for this study. The cumulative P(ID) for the 14 loci used was 1.2×10^{-9}, providing sufficient discriminatory power for differentiating individuals in our analyses (Waits et al., 2001). $P(ID)_{sib}$ was calculated to be less than 0.01 at 7 loci, and we therefore excluded from further analyses any samples which were successfully genotyped at fewer than 7 loci (n=14). We

also allowed identical samples which matched at 7 or more loci to differ at up to 1 locus to account for genotyping error. In total, we successfully genotyped 385 samples (96% genotyping success), which represented 162 unique individuals. 124 unique individuals were identified from KNP, belonging to 73 males and 51 females. 38 unique individuals were identified from BINP, belonging to 23 males, 14 females, and 1 individual of unknown sex.

Genetic Diversity

Genetic diversity values for KNP and BINP are presented in Table 1. The number of alleles per locus across both parks ranged from 2 at locus Lcy-M27 to 12 at locus FH71. On average, the elephants of KNP had 6.000 ± 0.663 SE unique alleles per locus and of BINP had 4.429 ± 0.429 SE alleles per locus. Two loci in KNP (FH71, LA5) and 1 locus in BINP (LafMS04) deviated significantly from HWE. The inbreeding coefficient (F) was negative for 11 of the 14 loci tested in BINP (mean: -0.095 ± 0.031 SE). This pattern is consistent with a population which has experienced outbreeding. In contrast, only 3 of 14 loci in KNP showed negative F values (mean: 0.049 ± 0.022 SE). KNP and BINP showed distinct clusters along PC1 using Principal Component Analysis (Figure 2), and pairwise F_{ST} between the two parks was 0.11.

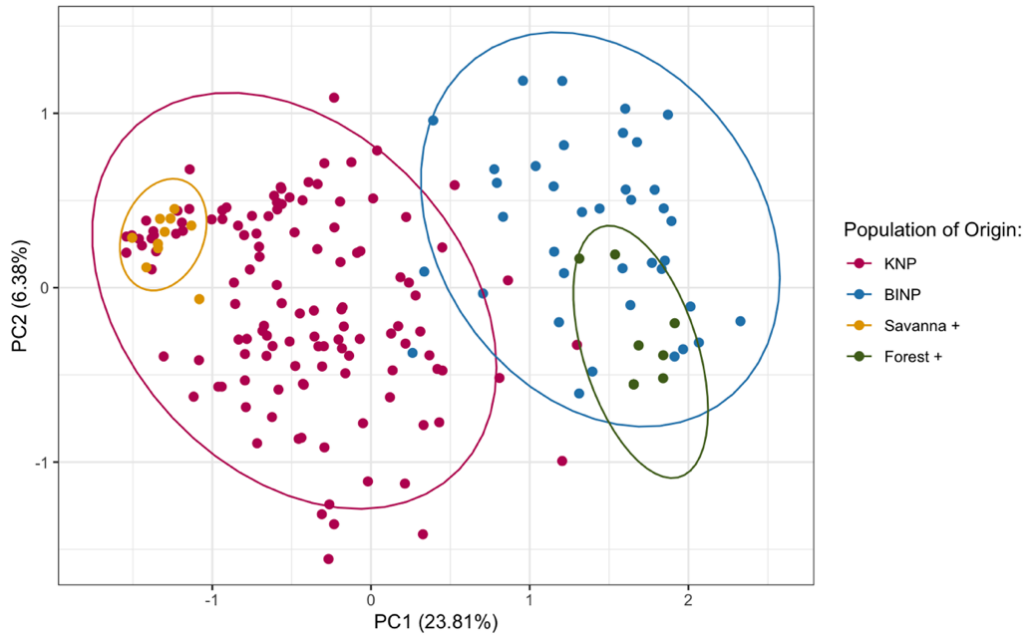


Figure 2. Ordination plot illustrating genetic distance between individuals computed using Principal Component Analysis from 14 microsatellite loci. Dots represent unique individuals, colored by population of origin (KNP, BINP, savanna elephant positive control, forest elephant positive control). Ellipses represent 95% confidence intervals for each group.

	BINP							KNP						
Locus	Na	Ne	Ho	He	uHe	F	HWE	Na	Ne	Ho	He	uHe	F	HWE
FH71	6.000	2.47	0.74	0.60	0.60	-0.24	ns	10.00	3.71	0.75	0.73	0.73	-0.03	P < 0.001
LA5	5.000	3.43	0.74	0.71	0.72	-0.04	ns	6.00	4.63	0.57	0.78	0.79	0.27	P < 0.001
LA6	5.000	1.89	0.47	0.47	0.48	0.00	ns	6.00	3.35	0.70	0.70	0.70	0.01	ns
LafMS03	7.000	3.69	0.83	0.73	0.74	-0.14	ns	6.00	2.69	0.59	0.63	0.63	0.06	ns
LafMS04	4.000	1.95	0.42	0.49	0.49	0.14	P < 0.01	6.00	2.19	0.50	0.54	0.55	0.09	ns
LcyM8	4.000	2.49	0.78	0.60	0.61	-0.31	ns	6.00	1.80	0.42	0.44	0.45	0.05	ns
LcyM16	7.000	3.83	0.82	0.74	0.75	-0.10	ns	11.00	4.20	0.74	0.76	0.76	0.03	ns
LcyM17	3.000	1.34	0.29	0.25	0.26	-0.14	ns	6.00	3.01	0.64	0.67	0.67	0.05	ns
LcyM26	3.000	2.43	0.65	0.59	0.60	-0.10	ns	7.00	1.91	0.44	0.48	0.48	0.07	ns
LcyM27	2.000	1.63	0.37	0.39	0.39	0.05	ns	2.00	1.03	0.03	0.03	0.03	-0.02	ns
LcyM29	4.000	3.18	0.76	0.69	0.69	-0.11	ns	5.00	1.68	0.39	0.41	0.41	0.04	ns
LcyM30	3.000	1.97	0.50	0.49	0.50	-0.02	ns	4.00	2.11	0.51	0.53	0.53	0.03	ns
LcyM40	3.000	1.71	0.50	0.41	0.42	-0.21	ns	2.00	1.17	0.16	0.15	0.15	-0.09	ns
LcyM44	6.000	4.22	0.84	0.76	0.77	-0.10	ns	7.00	3.36	0.62	0.70	0.70	0.12	ns
Mean	4.429	2.59	0.62	0.57	0.57	-0.09	-	6.00	2.63	0.50	0.54	0.54	0.05	-
SE	0.429	0.25	0.05	0.04	0.04	0.03	-	0.66	0.30	0.06	0.06	0.06	0.02	-

Table 1. Standard genetic diversity metrics for the elephants of KNP and BINP generated by GenAlEx v. 6.51b2. Abbreviations above are as follows: Na - Number of unique alleles; Ne - Effective number of alleles; Ho - Observed heterozygosity; He - Expected heterozygosity; uHe - Unbiased expected heterozygosity; F - Inbreeding coefficient; HWE - Deviations from Hardy Weinberg Equilibrium.

Census Results

We retained 180 captures for fecal DNA-based CMR analysis after excluding false recaptures (Table S2). Using capwire, the population estimate for KNP was 573 individuals (95% CI: 410 to 820) using the Equal Capture Model and 625 individuals (95% CI: 508 to 1036) using the Two Innate Rates Model. The likelihood ratio test showed no significant difference between these two models (L.R.=13.70, p=0.73, bootstraps=100). Given the park size of 795 km^2, crude elephant density was approximately 0.72 elephants/km^2 (95% CI: 0.52 to 1.03 elephants/km^2) using the ECM and 0.79 elephants/km^2 (95% CI: 0.64 to 1.30 elephants/km^2) using the TIRM. For BINP, the maximum likelihood population size point estimate was 96 individuals (95% CI: 64 to 145) using the Equal Capture Model and 108 individuals (95% CI: 86 to 173) using the Two Innate Rates Model. As in KNP, a likelihood ratio test showed no significant difference between these two models (L.R.=5.28, p=0.94, bootstraps=100). Crude elephant density for this 331 km^2 park was 0.29 elephants/km^2 (95% CI: 0.19 to 0.44 elephants/km^2) using the ECM and 0.33 elephants/km^2 (95% CI: 0.26 to 0.52 elephants/km^2) using the TIRM. Because no significant difference was detected between the ECM and TIRM models in either park, we report estimates generated using the simpler of the two models (ECM).

Species Identity

All STRUCTURE runs showed the strongest support for two clusters (K=2) with species-specific ancestry. Because each of our implemented models showed similar trends, we report results from the independent STRUCTURE run without location priors here. Results for each of the alternative models implemented are reported in the supplemental data (Figures S2-S3). Pure forest elephant positive controls and pure savanna elephant positive controls showed Q > 0.9 for

cluster I and cluster II respectively, while samples collected from the Western Ugandan hybrid zone showed a range of intermediate ancestries (Figure 3, Figure S1). Using a cutoff of Q>0.9, we classified elephants from the separate STRUCTURE runs as pure forest (cluster I), pure savanna (cluster II), or hybrid (intermediate). Of the 124 unique individuals detected in KNP, 22 elephants (17.7%) were classified as pure savanna, 1 elephant (0.8%) as pure forest, and 101 elephants (81.5%) as hybrid. In contrast, in BINP 33 elephants (86.8%) were classified as pure forest, while 5 elephants (13.2%) showed intermediate ancestry. No elephants in BINP were classified as pure savanna elephants.

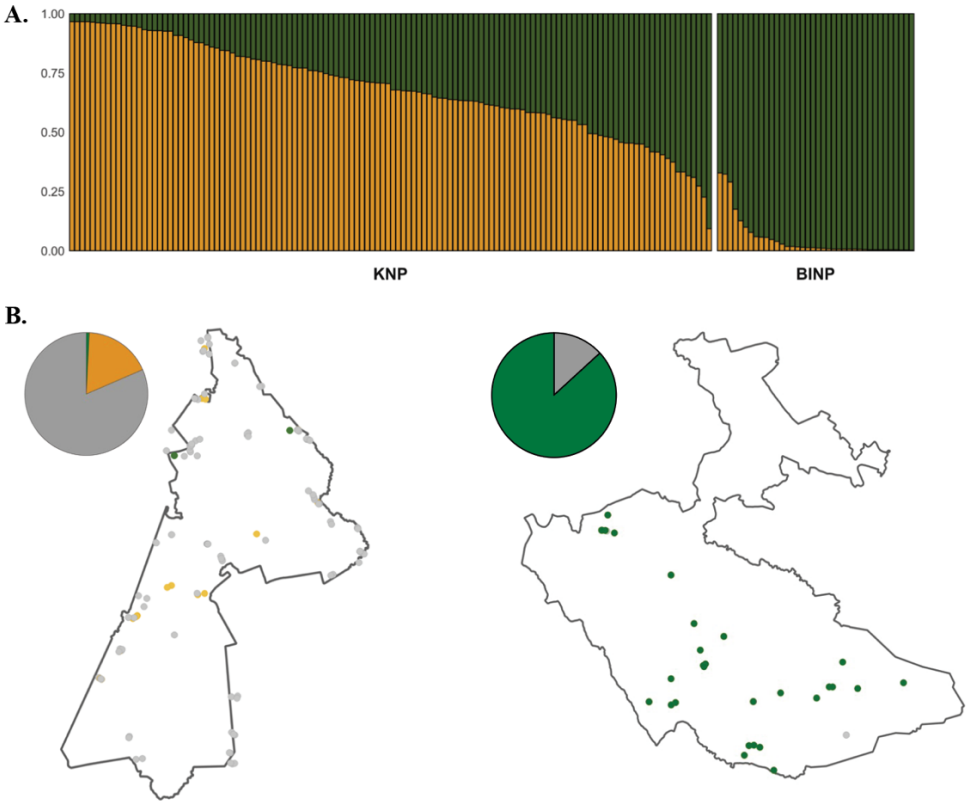


Figure 3. Species classifications for KNP and BINP using STRUCTURE. A) Plot showing ancestry proportions for the elephants of KNP and BINP inferred separately using the Admixture model in STRUCTURE (K=2) with no location prior. B) Elephant species distribution throughout KNP (left) and BINP (right), colored according to the highest likelihood elephant type (pure forest, pure savanna, hybrid) inferred using Q>0.9.

For our second species classification method, we used EBHybrids to classify individuals by species and to assign each sample a hybrid probability (Table 2). Using the highest stringency likelihood cutoff of 0.95 only one elephant (0.8%) in KNP was classified as pure forest, 49.2% as hybrid, 15.3% as pure savanna, and 34.7% as unclassified. At a cutoff of 0.95, the majority of the hybrids in this park were not further classified by hybrid type (86.9%). However, using a more permissive cutoff of 0.5, hybrids were further classified as either savanna backcrosses (85.4%) or F2 hybrids (14.6%). Using a cutoff of 0.95 in BINP, we find that 34.2% of the elephants in BINP classify as pure forest, 10.5% as hybrids, and 55.3% as unclassified. No pure savanna elephants were detected using this method. Hybrids in this park were all "unclassified" by hybrid type using a stringency of 0.95 but classified as primarily forest backcrosses (72.7%) using a stringency of 0.5.

	Species				Hybrid Classification				
Cutoff	Forest	Savanna	Hybrid	Unclassified	F1	F2	BXF	BXS	Unc
BINP									
EB: 0.95	13	0	4	21	0	0	0	0	4
EB: 0.8	22	0	7	9	0	0	3	0	4
EB: 0.5	27	0	11	0	3	0	8	0	0
Q > 0.9	33	0	5	0	-	-	-	-	-
KNP									
EB: 0.95	1	19	61	43	0	2	0	6	53
EB: 0.8	1	24	78	21	0	10	0	42	26
EB: 0.5	1	34	89	0	0	13	0	76	0
Q > 0.9	1	22	101	0	-	-	-	-	-

Table 2. Maximum likelihood species classifications for individuals in KNP and BINP, inferred using EBHybrids (EB: 0.95, 0.8, 0.5) and STRUCTURE (Q>0.9). Hybrid classifications generated by EBHybrids were: F1 hybrid (F1); F2 hybrid (F2); Forest elephant backcross (BXF); Savanna elephant backcross (BXS); Unclassified hybrid (Unc).

Discussion

This work represents the first high-resolution assessment of population size and species identity of African elephants living in forested protected areas embedded within a hybrid zone. Through pairing intensive park-wide survey efforts with a novel non-invasive HTAS framework, we corroborate previous findings which show pure forest, pure savanna, and hybrid elephants living in the region (Mondol, 2015; Kim & Wasser, 2019) while also highlighting that species identity strongly intersects with protected area. Despite the geographic proximity and landscape-scale similarities between these two forested parks, we demonstrate that the majority of elephants across KNP are hybrids (81.5%) with some pure savanna elephants (17.7%), while the majority of the elephants across BINP are pure forest elephants (86.8%) with some hybrids (13.2%). We integrate these findings within a fecal DNA-based CMR framework for elephants living in these two forested parks, and find a larger population with a higher elephant density per km^2 in KNP and a smaller and less dense, but potentially growing, elephant population in BINP. Taken together, these analyses demonstrate different management landscapes for the elephants in these two forested sites.

Kibale National Park

The population size and density estimates for KNP generated in this study (573 individuals, 95% CI: 410 to 820; 0.72 elephants/km^2) are remarkably consistent with the most recent size estimate for this park (566 individuals, 95% CI: 377 – 850; 0.71 elephants/km^2) which was generated using the traditional LTDS method (Aleper et al., 2021). The comparable point estimates and confidence interval overlap between these two studies together suggest a trend of population growth in this park over the past two decades (Aleper et al., 2021; UWA,

2016). Additionally, this work supports previous findings which suggest that fecal DNA-based CMR can perform comparably to the traditional LTDS method for surveying elephant populations in forested areas (Hedges et al., 2013; Laguardia et al., 2021a). Given the similar findings between these two methods, we argue that fecal DNA-based CMR censuses should be used for potentially admixed populations like this one, since this method provides species resolution information which lacks in the LTDS, and thus facilitates joint inference of species distribution, hybrid prevalence, and population abundance.

A recent study conducted in the Sebatoli region, a 25 km^2 area at the northernmost part of KNP, found that 81.3% of the elephants using this region were hybrids and the remaining 18.7% were pure savanna elephants (Bonnald et al., 2021). The absence of any pure forest elephant detections in this previous study was particularly notable given that KNP is a primarily forested park. However, because the study's surveyed region constitutes only ~3% of the park's total area and is comprised primarily of regenerating forest, it remained unclear whether these results were representative of the prevalence of elephant species throughout KNP. Our study expands upon this finding by extending our genetic survey across the park's entire area for the first time. Interestingly, we identify only one pure forest elephant out of the 124 unique individuals that we sampled (<1%), suggesting that this dearth of forest elephants extends throughout the entire park. As in the Sebatoli region, the majority of the elephants that we detected throughout the park were also hybrids (81.5%) and savanna elephants (17.7%).

These results validate the broad findings of Bonnald et al. (2021) and simultaneously suggest that elephant space use within the park does not strongly correlate to species-identity. Notably, the findings presented in this study also raise the unanswered question of how most of the KNP elephants could be hybrids given that so few forest elephants were detected. This

question is particularly important for the management of this population in light of the recent IUCN listing change. To this end, one important piece of evidence is that the majority of the hybrids that we detected were classified as savanna elephant backcrosses (85.4%) and F2 hybrids (14.6%), with no F1 hybrid detections. Taken together, this would suggest that the extensive hybridization detected in this park happened between pure parental types more than one generation ago, as the most recent generation in this park seems to be largely the offspring of either pure savanna elephants or of hybrids and savanna elephants. This population could therefore follow one of two trajectories, each with a different implication for the classification of the park's elephants: 1) Hybrid elephants could preferentially backcross to pure savanna elephants and/or savanna elephants could show increased reproductive success relative to hybrids. In this scenario, the elephant population of this park would show increasing savanna elephant ancestry with each generation, which would ultimately result in the loss of forest alleles from the gene pool (Schumer et al., 2017). If this were the case, then KNP might best be classified as a savanna elephant population for conservation and management purposes. Alternatively, 2) Hybrids in this park could mate randomly with respect to genetic background and/or not show decreased reproductive success relative to pure savanna elephants, which would ultimately lead to the formation and maintenance of a hybrid swarm (Morgan-Richards et al., 2021). In hybrid swarms, all individuals are admixed to varying degrees, which can lead to the effective genetic extinction of one or both parental species from a population (Allendorf et al., 2001). If this were the case, then KNP would be most appropriately classified as a hybrid population (CITES, 2022).

Bwindi Impenetrable National Park

The work presented in this study constitutes both the first park-scale, species-level assessment for the elephants of BINP and the first comprehensive elephant census for this park in three decades. The confidence intervals for our CMR model (ECM: 96 individuals, 95% CI 64 to 145; 0.29 elephants/km^2) suggest that more elephants are using this landscape than are captured by the current population estimate on record (UWA, 2016). The last systematic survey of the park's elephants (conducted 1992-1993, recce method) put this population at 22 individuals (0.07 elephants/km^2), including only one adult male, and attributed this small population size to intense poaching throughout the park in the late 1970s (Babaasa, 1994). The Uganda Wildlife Authority's current number on record for this park is 43 individuals, which is an estimate based on ranger encounter data (UWA, 2016). Considering each of these numbers, there are two possible interpretations for our results. First, the methodology used in Babaasa (1994) differed from that used in this study in that it relied solely on physical dung characteristics, and not genetic data. It is therefore possible that this number may have been an undercount of the park's elephants if the physical characteristics of individuals' dung at that time were similar enough to be indistinguishable. Alternatively, our results could indicate true population growth for the elephants of BINP over the last 27 years. This interpretation is supported by an annual trend of increased ranger encounter data from 1995 to 2012 (UWA, 2016), as well as by an increase in crop raiding incidents by elephants in recent years (Butynski, 1986; Natukunda, 2019), including at new locations around the park's boundaries (Bitariho et al., 2019).

This raises the question of whether the putative growth that is seen in BINP is intrinsic (due to births within the park) or extrinsic (due to immigration from nearby protected areas). It

has been noted that BINP was effectively cut off from the GVL by the late 1950s (Brooks & Buss, 1962; Plumptre et al., 2007), and that no migration corridors currently exist to facilitate recruitment to BINP from any of the three nearest neighboring elephant populations (Parc National des Volcans, Virunga National Park, Queen Elizabeth National Park) (Butynski, 1986; Plumptre et al., 2007). However, studies to date also suggest that forest elephants are characterized by intrinsic growth rates two to three times slower than those of savanna elephants (Turkalo et al., 2017). The Dzanga Bai forest elephant population in the Central African Republic, for example, has an average inter-birth interval of 68 months and a median primiparous age of 23 years, meaning that the doubling time for this population would fall between 41 and 60 years (Turkalo et al., 2017). Even assuming the most permissive doubling time (41 years), it remains unlikely that the substantial population increase noted in our work is entirely intrinsic. This would suggest: 1) that there has been some migration to this park from one of the three nearest elephant populations since 1992; 2) that the previous number on record was an undercount; or 3) some combination of these two explanations. Additionally, the presence of several recent hybrids within this park further suggests that although BINP is primarily a forest elephant population today (86.8% pure forest), savanna elephants (or hybrids) have contributed to this park's gene pool within the past few generations, most likely from the presumed last connection between BINP and the GVL through a corridor to Virunga National Park (Plumptre et al., 2007). Of the 14 microsatellites that we analyzed for this study, 11 loci showed signatures of outbreeding for this park, potentially lending support to this idea. Given the faster life histories of savanna elephants, with a median primiparous age of 11.2 - 14 years (Moss, 2001; Gough & Kerley, 2006; Foley & Faust, 2010; Wittemyer et al., 2013), it is possible that savanna elephants have also contributed to the faster than expected growth observed in this

population. However, our work suggests that further studies are needed to examine the degree to which the elephant population of BINP is truly isolated from the GVL, as well as the impacts of hybridization on the life history traits of hybrid elephants.

The Western Ugandan Hybrid Zone

The underlying cause of the unique modern-day hybrid zone between forest and savanna elephants in Western Uganda remains uncertain. It has been proposed that a recent history of poaching-induced decline throughout the 1970s and early 1980s in Uganda and beginning in the 1990s in the Eastern DRC has promoted a series of transboundary migrations for the elephants of this region, pushing elephant species outside of their typical ranges and resulting in the elevated levels of hybridization observed today (Mondol et al., 2015; Bonnald et al., 2021). When conspecifics are rare, Hubb's principle (alternatively called the "desperation hypothesis") states that the less frequent species will be more likely to mate with a heterospecific due to a scarcity of conspecific mates (Hubbs, 1955). Severe poaching pressure might therefore mediate hybridization by imposing population bottlenecks which reduce conspecifics. This idea has been demonstrated between naturally sympatric antelope species which showed elevated levels of hybridization following the Angolan civil war, when intense poaching severely reduced the population sizes of both species (Vaz Pinto, 2016). If this were also the case for Western Uganda, then this hybrid zone would be considered relatively young and mediated by poaching.

Alternatively, this hybrid zone could be more ancient. The presence of forest elephant derived (F-clade) mitochondrial DNA in pure savanna elephant populations throughout East and southern Africa (Debruyne, 2005; Eggert et al., 2002; Ishida et al., 2011, 2013; Johnson et al., 2007; Nyakaana et al., 2002) suggests that shifting ecotones over a longer, more ancient

timeframe may have resulted in range contractions and expansions for these two species, with limited hybridization and introgression occurring where the ranges meet. It is therefore possible that the hybrid zone observed in Western Uganda today is simply a modern-day window into elevated levels of hybridization which have followed a shifting forest-savanna transition zone through time (Oliveras & Malhi, 2016). These two hypotheses are not mutually exclusive, and testing them would require denser, genomic-scale data to characterize the history of admixture in this region (for example: Galaverni et al., 2017; Mattucci et al., 2019). However, the lack of F1 hybrids which we detected in this study, taken together with the prevalence of backcrossed hybrids in each park, suggests that a third potential driver of hybridization may also be at play in this region (Mondol et al., 2015).

Disturbance-mediated hybridization is characterized by a patchy, non-random distribution of hybrids and parental types across an anthropogenically altered landscape (Grabenstein & Taylor, 2018). Habitat conversion, in particular, can have context-dependent consequences for hybridization in different species (Urbanska et al., 1997; Li et al., 2017; Tovar-Sanchez & Oyama, 2004). For example, the process of converting land can remove barriers which were historically associated with facilitating or maintaining allopatric speciation, resulting in increased hybridization between previously-isolated species; this same process can also isolate closely-related species in fragments of land which are much smaller than their species-typical range sizes, promoting more frequent interaction and increased levels of hybridization (Grabenstein & Taylor, 2018; Hasselman et al., 2014). From the 1920s to the 1990s, the range of Uganda's elephants decreased from ~70% of the country's land mass to only ~7% (UWA, 2016); consequently, the country's elephants now persist in a series of fragmented protected areas embedded in a dense agricultural landscape (UWA, 2016; Brooks & Buss, 1962). Our results

indicate that two of these protected areas (KNP and BINP) show both high levels of genetic differentiation (F_{ST}=0.110) and contrasting species distributions and patterns of hybridization, as is characteristic of systems with disturbance-mediated hybridization (Grabenstein & Taylor, 2018).

These data suggest that the landscape-scale patterns of isolation and connectivity throughout the GVL might be interacting with other mechanisms (poaching-mediated hybridization and/or natural hybridization) to shape the patterns of hybridization that we observe in Western Uganda today. For instance, although BINP is currently thought to be cut off from the GVL, its last known connection was via a buffer zone between BINP and the southern sector of the DRC's Virunga National Park, a population which has been shown to harbor forest elephants (Groves & Grubb, 2000). While it is uncertain whether one or both elephant species historically ranged in BINP, it is possible that forest elephants from the DRC migrated into and/or interbred with the elephant population of this park before it was ultimately cut off from the remainder of the GVL. Unlike BINP, KNP is potentially still connected to the GVL via a corridor to Queen Elizabeth National Park (Figure 1), a protected area to its south which currently harbors primarily savanna elephants and hybrids (Kim & Wasser, 2019). Elephants historically undertook seasonal migrations between these parks (Wing & Buss, 1970), but the degree to which this corridor is used today remains unknown (Plumptre et al., 2008). As in BINP, it is unknown what species historically ranged in KNP, but this landscape-scale pattern could explain both the lack of forest elephants and the higher number of hybrids that we observe in KNP today.

Conservation and Management Implications

The recent decision by the IUCN to split the listing status of Africa's elephants highlights the differing threats of extinction faced by the two species, but also poses a series of new challenges including the need to understand how to classify and manage hybrid elephant populations like those in Western Uganda (Hart et al., 2021). Currently, technical guidance with respect to the classification of hybrid elephants is lacking from major conservation and regulatory authorities, including the IUCN and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Bauer et al., 2021). To this end, a taxonomy task force was recently established by the IUCN AfESG to address these concerns for Africa's elephants (Hart et al., 2021). While weighing in on these decisions is beyond the scope of this work, below we highlight several key findings about the hybrid zone in Western Uganda which might contribute to this conversation, as well as potential implications for conservation and management, and key areas for future investigation.

First, our work demonstrates that site-specific, species-level monitoring and management efforts will be critical to developing conservation policy for admixed elephant populations moving forward. This is because viewed as a whole, the elephants of Western Uganda show high levels of hybridization (Kim & Wasser, 2019; Mondol et al., 2015), leaving their listing status under the new IUCN guidelines uncertain (Hart et al., 2021). However, at the more granular level, we demonstrate that even within the largest-known modern hybrid zone between forest and savanna elephants, landscape-scale patterns of both species distribution and connectivity can inform on listing decisions. For example, because the elephants of BINP are primarily forest elephants (86.8%) and are currently isolated from other elephant populations within the GVL by habitat conversion, we argue that this park's elephants would most appropriately be classified as

a forest elephant population by the IUCN, and that their numbers should contribute to forest elephant counts in both national and international elephant inventories. In contrast, the elephants of KNP were classified as 81.5% hybrid and showed a hybrid index distribution consistent with that of a hybrid swarm (Figure S1). This population also putatively retains a connection to the GVL which harbors hybrids, savanna elephants and forest elephants. For these reasons, we suggest that given what is currently known about the elephants of KNP, this park would most appropriately be classified as a hybrid population (CITES, 2022). Under current guidelines, this would mean that the elephants of this population would not directly contribute to the IUCN's continent-wide inventories for either forest or savanna elephants, but that they would retain their current Appendix I protections given that CITES currently recognizes only one species – the "African elephant" (CITES, 2022). Hart et al. (2021) outline three alternative directions that CITES might now take following the updated IUCN listing status of Africa's elephants, noting that the organization could: 1) maintain its current listing and recognize only one species; 2) update its listing to "*Loxodonta spp.*" to directly acknowledge two species while explicitly retaining protections for hybrids; or 3) split its listing status for Africa's elephants to reflect two species. These options should be weighed carefully, given that the hybrid elephants of KNP would retain Appendix I listing status in the first two cases, but the third would leave hybrids without explicit CITES protections.

Second, at the national scale, we emphasize that Uganda is a range state for both forest and savanna elephants and should reflect this status as it develops the next iteration of the country's national elephant conservation management plan, which is currently set to expire in 2026 (UWA, 2016). Importantly, while our work generates critical data for both wildlife authority management of Uganda's elephants and the classification of these elephants in

continent-wide survey efforts, there are additional forested protected areas throughout Western Uganda (UWA, 2016) which face a time-sensitive demand for these same data. In contrast to the LTDS method, there is significant potential for the fecal DNA-based CMR method applied in our study to be smoothly integrated into pre-existing, regularly conducted park surveys by rangers on the ground. We suggest that this method be applied to jointly infer both census numbers and species distributions for elephants throughout these additional protected areas. This information would not only contribute to continent-wide elephant inventories, but could also facilitate new elephant tourism ventures by highlighting which elephant species can be found in which protected areas throughout the country. Ecotourism plays a critical role in funding Uganda's wildlife management (Guyson, 2021), and the notable presence of both elephant species within the country could both bring in additional revenue for the protection of the country's elephants and provide new opportunities for outreach about the different threats of extinction faced by forest and savanna elephants.

Finally, we propose that further work is needed to assess whether the on-the-ground management needs of hybrid elephants differ from one or both of their parental species. Specifically, further data examining the effects of hybridization on the behavior, health and fitness of elephants will prove critical to informing management efforts moving forward. Forest and savanna elephants have demonstrated species-characteristic resource requirements in their respective environments: forest elephants are highly frugivorous (Campos-Arceiz & Blake, 2011) and are dependent on localized saline bais to obtain dietary sodium (Vanleeuwe et al., 1997), while savanna elephants are primarily grazers and browsers (Cerling et al., 1999), and are frequently limited by water availability in the dry savanna woodlands in which they occur (Wittemyer et al., 2005). The extent to which these different resource requirements are species-

typical, as opposed to environmentally dictated, remains unclear. However, to understand the consequences of hybridization for the conservation and management of Africa's elephants, further work is needed to: 1) quantify the resource needs of hybrid elephants, and determine whether the protected areas in which they are located are sufficient to meet them; 2) examine how the health and fitness of hybrid elephants compares to their parentals in the region; 3) identify the underlying factors facilitating and/or maintaining hybridization in this region, especially with regard to whether the hybrid zone is natural or anthropogenically mediated; and 4) investigate putative prezygotic and postzygotic barriers to hybridization in this population, including evidence for behavioral isolation.

Elephant populations continue to decline across much of the African continent (Chase et al., 2016; Maisels et al., 2013; Poulsen et al., 2017), and a changing climate together with habitat conversion could change the way that African forest and savanna elephants interact in locations where their ranges overlap around the Congo Basin (Brandt et al., 2012; Dejene et al., 2021). For these reasons, a continued effort to monitor and understand the effects of hybridization on the elephants in the unique hybrid population of Western Uganda will be critical to continent-wide elephant conservation both today and in the future.

BRIDGE

In Chapter II, I applied non-invasive genetic monitoring techniques to generate a high-resolution assessment of elephant abundance and species identity for two IUCN priority sites in Western Uganda. I found that species identity strongly intersected with site, suggesting that landscape-scale factors influence the distribution and dynamics of hybridization that we observe between African forest and savanna elephants in the region. However, it remains unclear how these factors intersect with aspects of elephant sociality to shape the patterns of hybridization and species distribution that we observe today. In Chapter III, I build upon my findings from Chapter II to quantify the behavioral dynamics underlying this unique hybrid population. In particular, I characterize the social system of a hybrid elephant population for the first time by examining aspects of social structure, organization, and mating throughout the region.

CHAPTER III

PATTERNS OF MIXED-SPECIES ASSOCIATION AND HYBRIDIZATION IN THE ELEPHANTS OF BWINDI IMPENETRABLE AND KIBALE NATIONAL PARKS, UGANDA

Contributions

Claire K. Goodfellow, Nelson Ting, Daniella E. Chusyd, Caitlin P. Wells, Dennis Babaasa, Patrick A. Omeja, Charles Tumwesigye, Michael D. Wasserman, Colin A. Chapman, Richard Mutegeki, and Jena R. Hickey are co-authors on this chapter. Claire K. Goodfellow, Nelson Ting, Daniella E. Chusyd, and Caitlin P. Wells contributed to study design. Claire K. Goodfellow, Daniella E. Chusyd, Jena R. Hickey, Richard Mutegeki and Michael D. Wasserman coordinated sample collection efforts. Claire K. Goodfellow contributed to sample collection, and was responsible for all genetic lab work, data analyses, and the writing of this manuscript. Patrick A. Omeja, Dennis Babaasa, and Colin A. Chapman provided intellectual support.

Introduction

Sociality is a key component to survival for many species, and different social systems have been considered adaptive strategies in response to varying environmental contexts (Silk, 2007). Social systems, alternatively called "societies," are broadly comprised of three distinct components: social organization, social structure, and mating systems (Kappeler & van Schaik, 2002). Social organization encompasses the composition and stability of social groups, while social structure includes the types of social relationships that occur within those groups, and mating systems refer to the social and genetic patterns of mating in a society (Kappeler & van Schaik, 2002). Group living species derive a broad range of benefits from their characteristic

social systems which can include an increased ability to find and/or exploit resources (Packer & Ruttan, 1988; Johnson et al., 2002), a greater capacity to defend themselves and/or their young from predators (Hass & Valenzuela, 2002; Teunissen et al., 2021), assistance rearing young (Clutton-Brock et al., 2002; White et al., 2010), and knowledge sharing (Gager, 2018; McComb et al., 2001). However, there are also costs associated with group-living, most notably increased competition from conspecifics for limited resources (Janson & van Schaik, 1988; Isbell, 1991) and higher rates of disease transmission (Molvar & Bowyer, 1994; Chiyo et al., 2014; Romano et al., 2022). Context-dependent trade-offs between these costs and benefits have consequences for the evolution of different social systems (Majolo et al., 2008; Markham et al., 2017; Silk, 2007), and examining natural variation in social systems between closely related species can inform on the factors driving sociality in different contexts.

Africa's two extant elephant species – the forest (*Loxodonta cyclotis*) and savanna (*Loxodonta africana*) elephant – both exhibit complex social systems (Athira & Vidya, 2021; Vidya & Sukumar, 2005). These animals diverged 2.6 – 5.6 million years ago (Palkopoulou, 2018), and face different environmental constraints in their respective ranges. Savanna elephants primarily range throughout the dry, savanna woodlands of southern and East Africa, and it is thought that the social system of this species is specifically adapted to both provide protection from lions and minimize competition with conspecifics around seasonally limited water (Power et al., 2009; Joubert, 2006; Loveridge et al., 2006; Ruggiero, 1991). In contrast, forest elephants are endemic to West Africa and the dense forests of the Congo Basin where water is plentiful year-round and their young are not subject to predation by lions, but the patchy distribution and ephemeral nature of fruiting trees throughout the landscape can lead to competition with conspecifics over resources (Blake & Inkamba-Nkulu, 2004; White et al., 1993; Campos-Arceiz

& Blake, 2011). Although the social systems of savanna elephants are more thoroughly understood than those of forest elephants (Moss et al., 2019), available data suggest that both species live in matriarchal societies in which females associate with close kin, while males disperse at reproductive age and live either solitarily or in loose bachelor herds (Charif et al., 2005; Schuttler et al., 2014b). However, these species also exhibit species-characteristic differences in social system.

Savanna elephants live in complex, matriarchal societies wherein females exhibit a multi-tiered, fission-fusion social structure driven by seasonal shifts in resource availability which correlate with the wet and dry seasons (Wittemyer et al., 2005). Individuals consistently associate with a "core group" of highly related females year-round, and fusion along lines of relatedness to associate in larger, "bond groups" when resources are abundant (Wittemyer et al., 2005). The most inclusive level of organization in this species is the "clan," which consists of individuals which share a dry season home range. Core groups on average consist of 8-12 individuals which include 4-5 adult females and their dependent offspring, while clans can range up to several hundred individuals (Moss & Poole, 1983). In this species, males associate with their maternal core group until they reach puberty, at which point they disperse and range either solitarily or in loose bachelor herds (Moss & Poole, 1983; Poole, 1982). Bachelor herds – or groups composed entirely of adult males – are common in this species. For example, one study in the Amboseli population found that up to 63% of detected males were part of a bachelor herd, while only 12.5% were solitary (Chiyo et al., 2011). After puberty, it is uncommon for males of this species to associate with females, except when they are in musth (a rut-like behavior) and spend extended periods of time mate-guarding (Poole, 1989). There is a high variance in

reproductive success of males in this species, where older males in musth sire a higher proportion of offspring than younger, non-musth males (Hollister-Smith et al., 2007).

Current data suggest that forest elephants exhibit less complex social structures than savanna elephants (Schuttler et al., 2014b; Hedwig et al., 2021). Forest elephant females also live in stable core groups, but these groups are on average smaller and less connected (Schuttler et al., 2014b) than those of savanna elephant society, and most often consist of one adult female and her dependent offspring (Fishlock et al., 2008; Merz, 1986; Morgan & Lee, 2007; Munshi-South, 2011; Querouil et al., 1999; Schuttler et al., 2014b; Theuerkauf et al., 2000; White et al., 1993). Although this species does exhibit fission-fusion dynamics, to date there has been no evidence for the complex, multi-level social structure which is characteristic of savanna elephant populations (Schuttler et al., 2014b; Fishlock & Lee, 2013). The large bachelor herds characteristic of savanna elephants have also not been demonstrated in forest elephants: in the well-studied Dzanga Bai forest elephant population, males were only sighted in multi-male groups 0.3% of the time (Turkalo et al., 2013). The mating system of forest elephants is very poorly understood, though there have been some suggestions that there is less variance in the reproductive success of males in this species (Roca et al., 2019).

Elephant behavior is highly flexible, and it is unclear the degree to which the variation in social systems between these two species is truly species-typical, as opposed to environmentally constrained. For instance, a unique population of savanna elephants in Namibia occur in small groups, more comparable in size to those of forest elephants than savanna elephants. It is thought that the unusual social system observed in this population is a consequence of the extreme environmental constraints of their desert environment (Legget et al., 2003). Alternatively, limited evidence has also suggested that forest and hybrid elephants may form larger groups, more

comparable in size to those of savanna elephants, when they are distributed in open savanna environments (Meyer, 2022). Other elephant populations have been shown to depart from their species-typical social organization in response to human disturbance. For example, unrelated females from different matrilines are known to associate within the same core social group following intense poaching pressure on a population (Gobush et al., 2009; Nyakaana et al., 2001; Archie et al., 2012), and possibly due to habitat modification (Brand et al., 2020).

Hybrid zones provide a unique opportunity for insight into the drivers of sociality, as they are a "natural laboratory" in which animals with varying genetic ancestries are subject to the constraints of a common environment (Barton & Hewitt, 1985; Hewitt, 1988). In particular, various aspects of sociality can follow one of three trajectories in a hybrid population: 1) hybrid individuals can exhibit one or more behaviors which are inconsistent with either parental species (Barrera-Guzman et al., 2017; Amaral et al., 2014); 2) hybrid individuals can exhibit traits which are intermediate between the species-typical traits of their parentals (Bergman et al., 2008; Pearson & Rohwer, 2000; Moore et al., 2010; Carli, 2022; Delmore & Irwin, 2014); or 3) hybrid sociality can skew toward traits associated with the social system of only one parental species (Bergman & Beehner, 2004). By studying social systems in hybrid populations, it is therefore possible to disentangle the varying constraints of both environment and phylogeny on various aspects of sociality.

Although the behavioral differences between African forest and savanna elephants have been documented in isolation, no work to date has examined the social dynamics of mixed-species elephant populations or the role that genetic ancestry plays in maintaining species-typical behavioral phenotypes. In this study, we examine the social group composition and species associations of elephants living in an African elephant hybrid zone for the first time. We

combine social network analyses with sequencing data from biparentally and uniparentally inherited genetic markers to elucidate how species identity influences patterns of social behavior at the group level. Specifically, we seek to address the following three questions related to the social systems of a hybrid elephant population: 1) How does genetic ancestry influence group membership and size? 2) Is the matrilineal social structure characteristic of African elephants maintained in a hybrid zone? 3) Is mating in this hybrid zone symmetrical, or is it biased with respect to genetic ancestry? Because both African elephant species exhibit matrilineal sociality, but it is thought that female forest elephants form smaller groups than female savanna elephants, we hypothesize that matrilineal social organization will be maintained in this hybrid population but that female group size will vary as a function of genetic ancestry. Furthermore, because forest elephant males are believed to be more solitary than savanna elephant males, we predict that forest males will be detected solitarily more frequently than hybrid or savanna males, and that bachelor herd size will vary as a function of genetic ancestry. Finally, because savanna elephant males are larger and presumably more competitively dominant than forest elephant males (Roca, 2019), we hypothesize that mating in this hybrid zone is largely driven by savanna elephant males mating with forest elephant females, and that hybrid individuals will therefore primarily carry savanna elephant Y chromosomes (paternally inherited) and forest elephant mitochondrial DNA (maternally inherited).

Methods

Study System and Sample Collection

This study was conducted in Western Uganda, a region which represents the largest known modern hybrid zone between African forest (*Loxodonta cyclotis*) and savanna (*L.*

africana) elephants (Kim & Wasser, 2019; Mondol et al., 2015). Sampling was conducted across two forested national parks in this region which have been demonstrated to harbor elephants of varying genetic backgrounds: Bwindi Impenetrable National Park and Kibale National Park. Bwindi Impenetrable National Park (BINP; 0°53' to 1°08' S; 29°35' to 29°50' E) is a 331 km^2 protected area in southwest Uganda which is characterized by medium-altitude moist evergreen forest and high-altitude submontane forest (Langdale-Brown et al., 1964). Both pure forest elephants and hybrids have been detected in this park (*Chapter 2*; Mondol et al., 2015). Kibale National Park (KNP; 0°13'-0°41'N and 30°19'- 30°32' E) is a 795 km^2 protected area which lies approximately 140 km to the north of BINP. This park is characterized by medium-altitude moist evergreen forest, medium-altitude moist semi-deciduous forest, moist acacia savanna, and forest-savanna mosaic habitat (Chapman & Lambert, 2000; Langdale-Brown et al., 1964), and has been shown to harbor primarily savanna elephants and hybrids, though a small number of forest elephants have also been detected (*Chapter 2*; Bonnald et al., 2022; Kim & Wasser, 2019; Mondol et al., 2015).

From October 2018 through August 2021, field teams consisting of 2-5 individuals systematically surveyed BINP and KNP for elephant dung using known elephant hotspots, elephant tracks, and community informants. When fresh dung piles (< 48 hours) were encountered, they were georeferenced and genetic samples were collected from the external surface of each distinct pile in one of two ways: 1) by placing 15 mL of fecal matter taken from the external surface of a bolus into 15 mL of RNAlater, or 2) by swabbing the external surface of a bolus using an Isohelix buccal swab, which was then stored in 1.5 mL of RNAlater. In cases where multiple dung piles were found at the same location, each distinct pile was individually sampled to capture associations between individuals. This dense sampling method prioritizes

capture of elephant associations at the cost of significant expected resampling of the same individual on the same day. In total, 399 samples were collected and analyzed for this study (BINP, n=143; KNP, n=256).

Genetic Analyses

DNA from each sample was extracted and genotyped as described in *Chapter 2* of this dissertation. Extractions were performed using the QIAamp Fast DNA Stool Mini Kit (Qiagen), with modifications to the manufacturer's protocol (*Chapter 2*; Archie et al., 2003). Samples were then molecularly sexed using a panel of three sex-linked markers (PLP, AMELY, SRY) following the protocol of Ahlering et al. (2011) and were genotyped at 14 microsatellite loci using a high-throughput amplicon sequencing approach on the Illumina MiSeq (see *Chapter 2* for detailed protocol). Homozygous genotype calls were confirmed in at least three independent replicates, and heterozygous calls were confirmed in two. Samples which were successfully genotyped at a minimum of 7 microsatellite loci were classified to individual and species as described in *Chapter 2*. Briefly, species assignments were made alongside a set of positive controls (*L. cyclotis*, n=9; *L. africana*, n=10) using STRUCTURE v. 2.3.4 (Pritchard et al., 2000) at K=2. Individuals which showed a posterior ancestry proportion (Q) of 0.9 or greater were classified as a "pure" species for that cluster, as has been previously done (Vähä & Primmer, 2006; Bohling & Waits 2011; Latch et al., 2011; Beaumont et al., 2001; Barilani et al., 2007; Trigo et al., 2008; Sanz et al., 2009). Due to high levels of differentiation between the elephants of BINP and KNP (F_{ST}=0.110), these parks were treated as two separate populations for genetic analyses. GenAlEx version 6.51b2 was used to identify unique individuals. Pairwise relatedness values between unique individuals were generated using both the Queller-Goodnight index in

GenAlEx and a maximum-likelihood relatedness index in MLRelate (Queller & Goodnight, 1989; Kalinowski et al., 2006; Peakall & Smouse, 2006).

To examine patterns of mating in this population, uniparentally inherited loci were sequenced for each unique individual. A 319 bp region of the mitochondrial control region (Brandt et al., 2012) was amplified for all individuals in 25 uL reaction volumes consisting of 12.5 uL Green GoTaq 2X Mastermix, 0.33 uM primers CR-F1 and CR-R2, 0.5 uL BSA, 7.34 uL H2O, and 3 uL DNA. Amplification conditions for this reaction consisted of an initial 3-minute denaturation at 95°C, followed by 35 cycles of 95°C for 30 seconds, 50°C for 45 seconds, and 72°C for 30 seconds, and a final extension step of 72°C for 5 minutes. A 719 bp region of the Y-linked, intronic ameloglobin gene was additionally amplified in two separate reactions for all individuals which were molecularly sexed as males. Each reaction consisted of 10 uL Qiagen Multiplex Mastermix, 6 uL H_2O, 0.2 uL BSA, 0.4 uL each of 10X forward and reverse primers (Reaction 1: AmelY-F1/AmelY-R1; Reaction 2: AmelY-F2/AmelY-R2; Mondol, 2015), and 3 uL of DNA. Amplification conditions consisted of an initial denaturation at 94°C for 15 minutes, followed by 40 cycles of 94°C denaturation for 30 seconds, 58°C annealing for 45 seconds, and 72°C extension for 45 seconds, and one final extension step of 72°C for 30 minutes. For each uniparental marker, amplification success was confirmed on a 2% agarose gel, and PCR product which showed a strong band at the expected size range was subjected to an enzymatic cleanup using ExoSAP-IT (Thermofisher) and submitted to Genewiz for Sanger sequencing.

Sequences were trimmed, assembled and aligned using Geneious v.2023.1 (Kearse et al., 2012). Each individual's mtDNA was classified as either "S-clade" or "F-clade" based on a set of species-diagnostic SNPs within the 319 bp control region (Ishida et al., 2013). These classifications represent deeply diverged clades which derived ~5.5 million years ago from the

savanna elephant and forest lineages, respectively, and have been widely used in the genus *Loxodonta* to examine patterns of maternal inheritance (Ahlering et al., 2012; Brandt et al., 2012; Debruyne, 2005; Lohay et al., 2020; Roca et al., 2001). Unique mitochondrial haplotypes were then visualized using the R package pegas v. 1.2 (Figure S3.1) and searched using the *Loxodonta Localizer* database (Zhao et al., 2019) to identify any previous detections of the haplotype in publicly available datasets and consistently assign haplotype nomenclature (Table S3.1). The Y chromosome was similarly classified as either "forest-typical" or "savanna-typical" based on species-diagnostic SNPs in the 719 bp ameloglobin gene. This is an intronic gene on the Y chromosome which can be used to examine patterns of paternal species inheritance in African elephants (Mondol et al., 2015). We performed Fisher's exact tests to test for differences in discordance between uniparental markers and the biparentally-inherited nuclear genome across sexes and locations.

Social Network Construction

In total, we analyzed 156 unique individuals which consisted of 34 pure forest elephants, 22 pure savanna elephants, and 100 hybrids. 38 individuals were analyzed from BINP, 33 of which were classified as forest elephants and 5 as hybrids. 118 individuals were analyzed from KNP, 22 of which were classified as savanna elephants, 1 as a forest elephant, and 95 as hybrids. Pairwise distances between all georeferenced samples were generated using the R package geodist v.0.0.8. In line with previous work (Brand et al., 2020; Munshi-South, 2011; Schuttler et al., 2014a), we considered samples to be "in association" if they were collected on the same day, judged to be deposited at approximately the same time, and found within a set distance from each other. Because elephant association has been defined at varying distances across studies, we

constructed social networks using four different association distances: 75 meters (Brand et al., 2020), 100 meters (Brand et al., 2020; Munshi-South, 2011), 250 meters (Brand et al., 2020; Schuttler et al., 2014a), and 500 meters (Wittemyer et al., 2005).

Social networks were constructed using the R package igraph v.1.4.1, with unique individuals as nodes and associations between individuals as edges. Nodes which are connected to each other, but not to the rest of the network, are referred to as "components." Edges between nodes were weighted by relatedness using the Queller-Goodnight R value, with thicker edges indicating more related individuals. We calculated the standard network metrics mean degree, edge density, mean distance, and clustering coefficient using igraph. Mean degree is the average number of edges to which each node is connected; edge density represents the ratio of edges which exist between nodes to all possible edges; mean distance represents the average shortest path from one node to another; and clustering coefficient represents the degree to which nodes in a network tend to cluster together (Whitehead, 2008). We generated three different networks from our data: 1) representing females only (hereafter, "female network"); 2) representing males only (hereafter, "male network"); and 2) representing all unique individuals (hereafter, "global network").

Social Network Analysis

Network analyses of hybrid populations can be used to test for correlations between phenotypic and/or genotypic variation and social behavior, which in turn can inform upon fine-scale patterns within social systems (Rowley, 2021; Safran et al., 2019; Zonana et al., 2019). Within our networks, we classified each component as monospecific (composed entirely of the same species type), heterospecific (composed of more than one species type), or hybrid

(composed of entirely hybrid individuals) and calculated the average size of each component type. We also calculated the average component size for each of our networks (global, female, male) and quantified the "average forest ancestry" for each component using ancestry-specific cluster values output from STRUCTURE at K=2 (*Chapter 2*). In order to test the hypothesis that group size will vary as a function of genetic ancestry, Poisson regressions were used to test for a correlation between component size and the average genetic ancestry of that component in each network. Additionally, to test the hypothesis that male forest elephants are more likely to be solitary than male hybrid and savanna elephants, we used a chi-squared test for differences in the observed frequency of solitary males across species types.

Finally, to examine whether aspects of matrilineal social organization are maintained in a hybrid population, we calculated the average relatedness within and between components using the Queller-Goodnight index. We also calculated coefficients of assortativity for each network in igraph using two different nominal variables: mtDNA haplotype and species type. Using this metric, a coefficient close to 1.0 represents increased association between phenotypically or genotypically similar individuals, while a proportion close to -1.0 represents avoidance of associations with such individuals. An assortativity coefficient of 0 represents no preference for or against associations based on similarity. Using this method, networks with high coefficients of assortativity by mtDNA haplotype would be considered to fit the expectation of female matrilineal social organization, while networks with high assortativity by species type (and not by mtDNA haplotype) would suggest that preference for association with conspecifics over heterospecifics outweighs matrilineal social organization in a mixed-species population.

Results

Social Network Descriptive Statistics

Network diagrams constructed using different association distances (75 meters, 100 meters, 250 meters, 500 meters) yielded largely similar results across all analyses, with minimal changes to component composition across distance. We therefore report on networks constructed using the most permissive cutoff of 500 meters. Networks constructed using alternate association distances of 250 meters, 100 meters and 75 meters are reported in the supplemental data (Appendix B, Figures S3.2 - S3.4). At the 500-meter association distance, our global network consisted of 156 nodes, 274 edges, 24 components and 27 singletons. Standard network statistics indicated that this population consists of many disconnected but strongly-clustered components: edge density and mean distance were both low across all networks (density: 0.01 - 0.08; distance: 1.00 – 2.03), but the clustering coefficient was high (0.67 – 1.00). However, the male network had a lower clustering coefficient (BINP: 0.67; KNP: 0.73) than the female network (BINP: 1.00; KNP: 0.97), suggesting that males form looser groups than females in this population. The largest component was detected in KNP and consisted of 20 individuals. Additional network statistics are presented in Table 1 and Table S3.2.

Network	N	Nodes	Edges	Singletons	Components	Largest Component	Mean Component Size	
							With Singletons	No Singletons
Global								
All	51	156	274	27	24	20	3.1 SD 4.0	5.4 SD 4.8
Forest	15	27	17	9	6	4	1.8 SD 1.2	3.0 SD 0.9
Savanna	5	11	16	3	2	6	2.2 SD 2.2	4.0 SD 2.8
Hybrid	25	57	73	15	10	9	2.3 SD 2.0	4.2 SD 1.9
Mixed	6	61	168	NA	6	20	NA	10.2 SD 7.8
Female								
All	21	64	95	7	14	9	3.0 SD 2.2	4.1 SD 1.9
Forest	5	8	4	3	2	3	1.6 SD 0.9	2.5 SD 0.7
Savanna	2	5	6	1	1	4	2.5 SD 2.1	4.0 SD 0.0
Hybrid	10	26	31	3	7	5	2.6 SD 1.5	3.3 SD 1.3
Mixed	4	25	54	NA	4	9	NA	6.3 SD 1.9
Male								
All	49	91	75	34	15	19	1.9 SD 2.6	3.8 SD 4.3
Forest	14	22	10	10	4	4	1.6 SD 1.0	3.0 SD 0.8
Savanna	5	6	1	4	1	2	1.2 SD 0.5	2.0 SD 0.0
Hybrid	27	39	14	20	7	4	1.4 SD 0.8	2.7 SD 0.8
Mixed	3	24	50	NA	3	19	NA	8.0 SD 9.5

Table 1. Network statistics for the global, female, and male networks. "N" indicates the sum of singletons and components detected within each network, and networks are further broken down by component type: entirely forest, entirely savanna, entirely hybrid, or mixed-species. Network statistic definitions are provided in the methods section above.

Component Composition, Assortativity, and Size by Species

Elephants of all three types (forest, savanna, hybrid) were detected within components in the global network (Figure 1). Hybrid components were the most frequently detected type (41.7%), followed by monospecific components (33.3%) and then heterospecific components (25%). Only one component, a large bachelor herd detected in KNP, captured all three elephant types. Because individuals from BINP and KNP are unlikely to be found in association due to spatial separation between the two parks, we subset our assortativity analyses by park. Overall, we detected low levels of assortativity based on species identity (BINP: 0.01; KNP: 0.29).

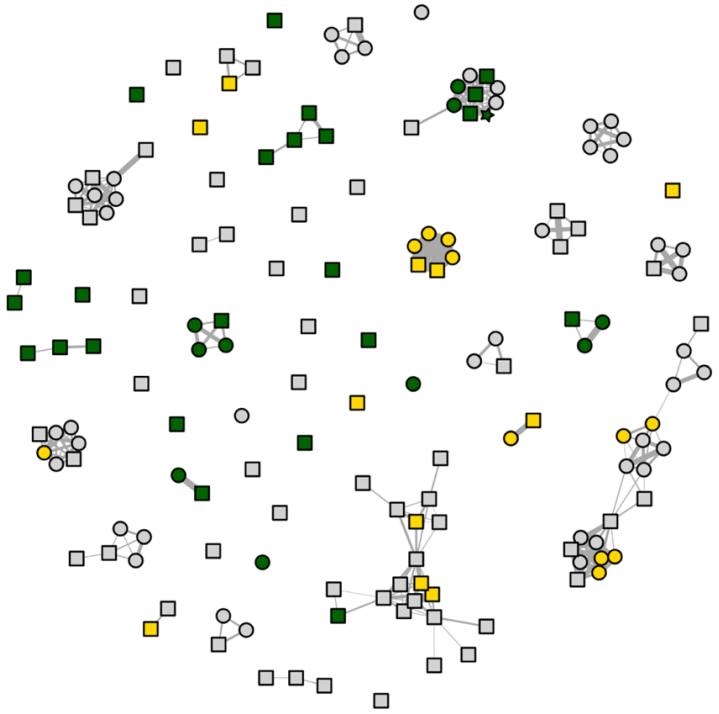


Figure 1. Global network by species identity. Elephants of all three types (forest, savanna, hybrid) were detected in mixed-species components within this network. Nodes represent all unique individuals detected in this study, while edges represent associations detected using a 500-meter association distance. Node shape denotes sex (circle: female, square: male, star: unknown) and edge weights represent relatedness between individuals, with wider lines indicating more closely related individuals. Node color represents species classification based on 14 microsatellite loci (yellow=savanna, green=forest, gray=hybrid).

Sex-based differences in sociality were also evident in our networks: 93.8% of females were found in components with more than one individual, while 85.2% of the detected singletons were male. Despite constituting the majority of singletons, most males detected in this study (75%) were not solitary, and forest males were no more likely to be detected solitarily than were savanna males ($\chi 2=0.53$, df=1, p=0.767). Females in both parks showed low levels of assortativity by species (BINP: 0.14; KNP: 0.31), while the males in KNP showed slight disassortativity by species (-0.01). No assortativity coefficient was calculated for the male network of BINP because only one hybrid male was detected in the park.

In the global network, the average component size for forest elephants (excluding singletons) was 3.0 ± SD 0.9, for savanna elephants was 4.0 ± SD 2.8, and for hybrid groups was 4.2 ± SD 1.9. Mixed-species components were the largest at 10.2 ± SD 7.8 individuals. Both the female and male networks showed a similar trend, with mixed-species components being on average the largest across all networks (Table 1). A significant negative correlation was detected between the average proportion of forest elephant ancestry of a component and the size of that component for the female network (p=0.0468), but this trend was not apparent in the male network (p=0.557) (Figure 2).

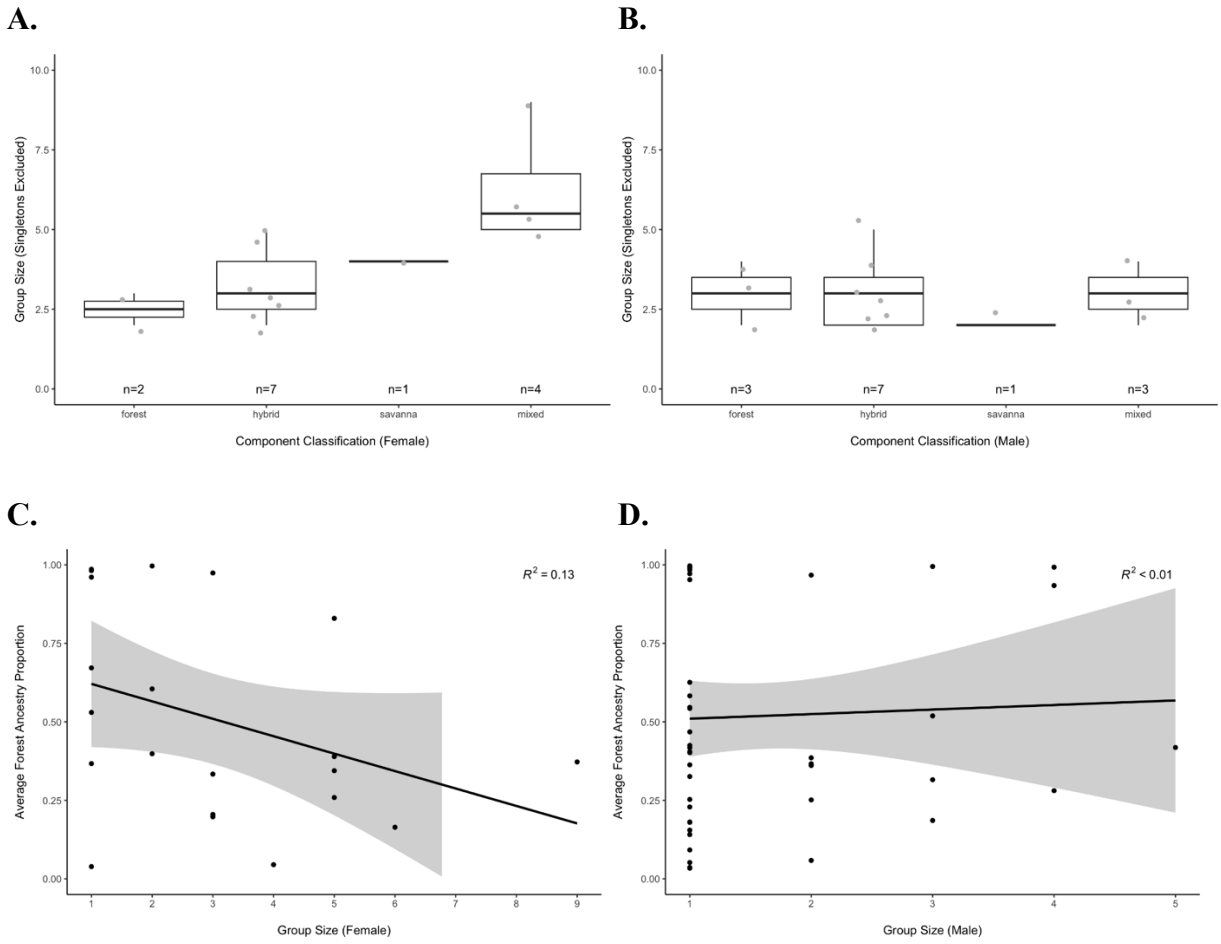


Figure 2. Component type vs. component size for the A) female and B) male networks, after excluding singletons from analyses. Group size vs. forest ancestry proportion derived from STRUCTURE at K=2 for the C) female and D) male networks. There is a negative correlation between the average forest elephant ancestry in a component and the size of that component for the female (p=0.0468), but not the male network (p=0.557). One outlier (a male, mixed species component with 20 individuals) was excluded from the graphs above, but analyses with and without this outlier yielded similar results. Graphs including outliers for all networks are presented in Figure S3.5.

Group Relatedness and Mitochondrial Haplotype

We found that female's mitochondrial haplotypes were correlated within each component, as 85.7% (n=12 of 14) of the components in our female network carried only one mitochondrial haplotype (Figure 3). Females in KNP were highly assortative by mitochondrial haplotype (0.72), while no assortativity coefficient was calculated for the female network of BINP because all detected females in this park shared the same mitochondrial haplotype

(LL062). In contrast, males were only slightly assortative by mitochondrial haplotype in both parks (BINP: 0.04; KNP: 0.02; Table 2).

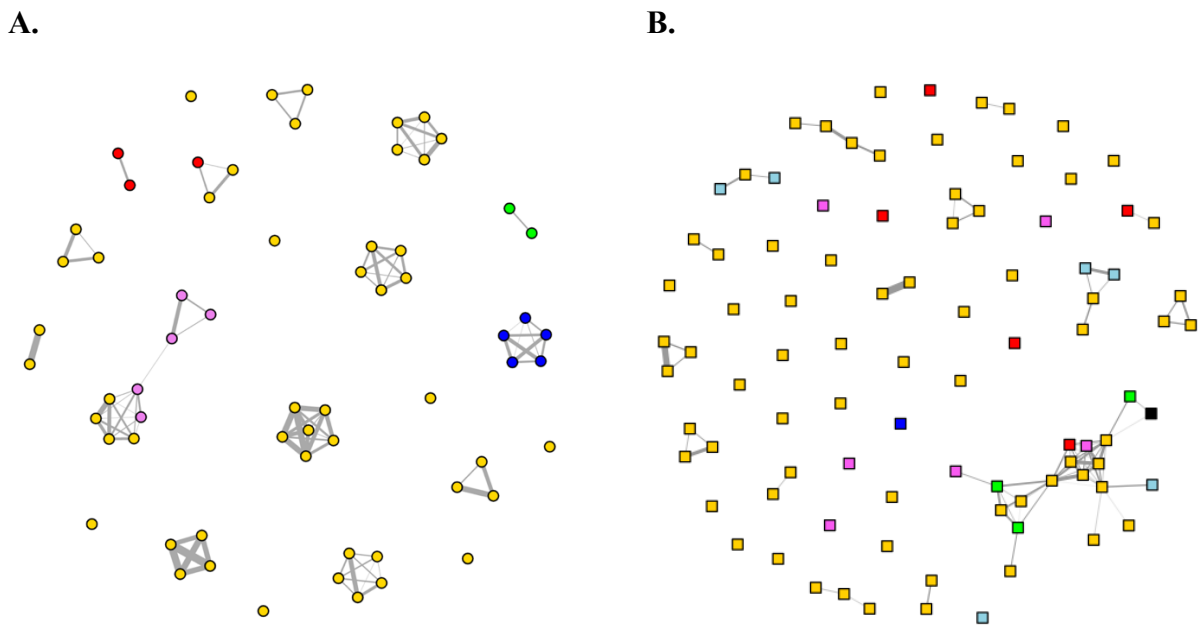


Figure 3. A) Female and B) Male networks colored by mitochondrial haplotype. Female's mitochondrial haplotypes are correlated within component, but this trend is not apparent in the male network. Nodes represent unique individuals detected in this study, while edges represent associations detected using a 500-meter association distance. Edge weights represent relatedness between individuals, with wider lines indicating more closely related individuals. Node color represents unique mitochondrial haplotypes for 319 bp of the mitochondrial control region (Yellow: LL062, blue: CG001, pink: LL068, red: LL069, green: LL027).

We found differences in relatedness within and between components across our networks. Average relatedness for the females within components in BINP was 0.29 ± SD 0.17 (between components: -0.13 ± 0.21) and in KNP was 0.15 ± SD 0.19 (between components: 0.01 ± 0.25). Male relatedness within components was lower than female relatedness within components across both parks (Table 2).

	Assortativity Coefficient		Avg Component Relatedness	
Sex	mtDNA	Species	Within	Between
KNP				
All	0.47	0.29	0.06 SD 0.25	-0.01 SD 0.24
Males	0.02	-0.01	-0.01 SD 0.29	-0.02 SD 0.23
Females	0.72	0.31	0.15 SD 0.19	0.01 SD 0.25
BINP				
All	0.29	0.01	0.12 SD 0.26	-0.04 SD 0.25
Males	0.04	NA	0.00 SD 0.17	0.05 SD 0.25
Females	NA	0.14	0.29 SD 0.17	-0.13 SD 0.21

Table 2. Coefficients of assortativity and relatedness for the three networks in this study. All networks are more assortative by mitochondrial haplotype than by species identity, although this pattern is more strongly apparent in the female network than the male network. Relatedness is also on average higher within components than between components for the female and global networks, but this trend is not apparent in the male network.

Maternal Genetic Ancestry

In individuals classified as hybrids, 79% of individuals carried S-clade mtDNA (F-clade: n=21 of 100; S-clade: n=79 of 100). This proportion did not vary significantly between sexes (Fisher's exact test: p=0.63) or parks (Fisher's exact test: p=0.58). Cytonuclear discordance, which is when an individual possesses the mitochondrial genome of one species but the nuclear genome of another, was also detected in 30 of 56 putatively "pure" individuals in this study (Figure 4). Of these, 28 of 30 cases (93%) of cytonuclear discordance consisted of putatively pure forest elephants carrying S-clade mtDNA. Females showed discordance more frequently than males, but this difference was not statistically significant (Fisher's exact test p=0.78).

Notably, patterns of cytonuclear discordance significantly differed across the two parks sampled in this study (Fisher's exact test: p<0.001). In BINP, cytonuclear discordance was observed in 28 of 33 putatively pure individuals, all of which were classified as pure forest elephants. The 5 cases of concordance between the mitochondrial and nuclear genome were all in males which carried F-clade mtDNA and a forest-typical nuclear genome. No females

detected in this park carried mtDNA haplotypes concordant with their nuclear genome. In contrast, cytonuclear discordance was only observed in 2 of 23 putatively pure individuals in KNP, both of which were savanna females carrying F-clade mtDNA. The remaining putatively pure savanna elephants in this park all carried S-clade mtDNA, while the one pure forest elephant detected in this park was a male carrying F-clade mtDNA. Overall, females exhibited cytonuclear discordance slightly more often than males, but this difference was not statistically significant in either park (Fisher's exact test: BINP p=0.22; KNP p=0.22).

Paternal Genetic Ancestry

We detected hybrid males carrying all four combinations of uniparental markers (Table 3). Of these, 60% carried a forest-typical Y chromosome, and the remaining 40% carried the savanna-typical haplotype (forest-typical: n=33 of 55; savanna-typical: n=22 of 55).

Mitochondrial DNA	Y Chromosome	Hybrid Count
Forest	Forest	5
Forest	Savanna	6
Savanna	Forest	28
Savanna	Savanna	16

Table 3. All four uniparental marker combinations are found in hybrid males in this study indicating that hybridization is bidirectional.

We did not detect any cases of discordance between the Y chromosome and the nuclear genome for putatively pure males in this study: 31 of 31 males carried the species-typical Y chromosome haplotype consistent with their species identity. However, the distribution of Y chromosome haplotypes in both putatively pure and hybrid males differed significantly across parks (Fisher's exact test: $p < 0.001$). In BINP 100% of sequenced males carried the forest-

typical Y chromosome (forest-typical: 20 of 20; savanna-typical: 0 of 20), while in KNP 50% carried a forest-typical and 50% carried a savanna-typical Y chromosome (forest-typical: n=33 of 66; savanna-typical: n=33 of 66).

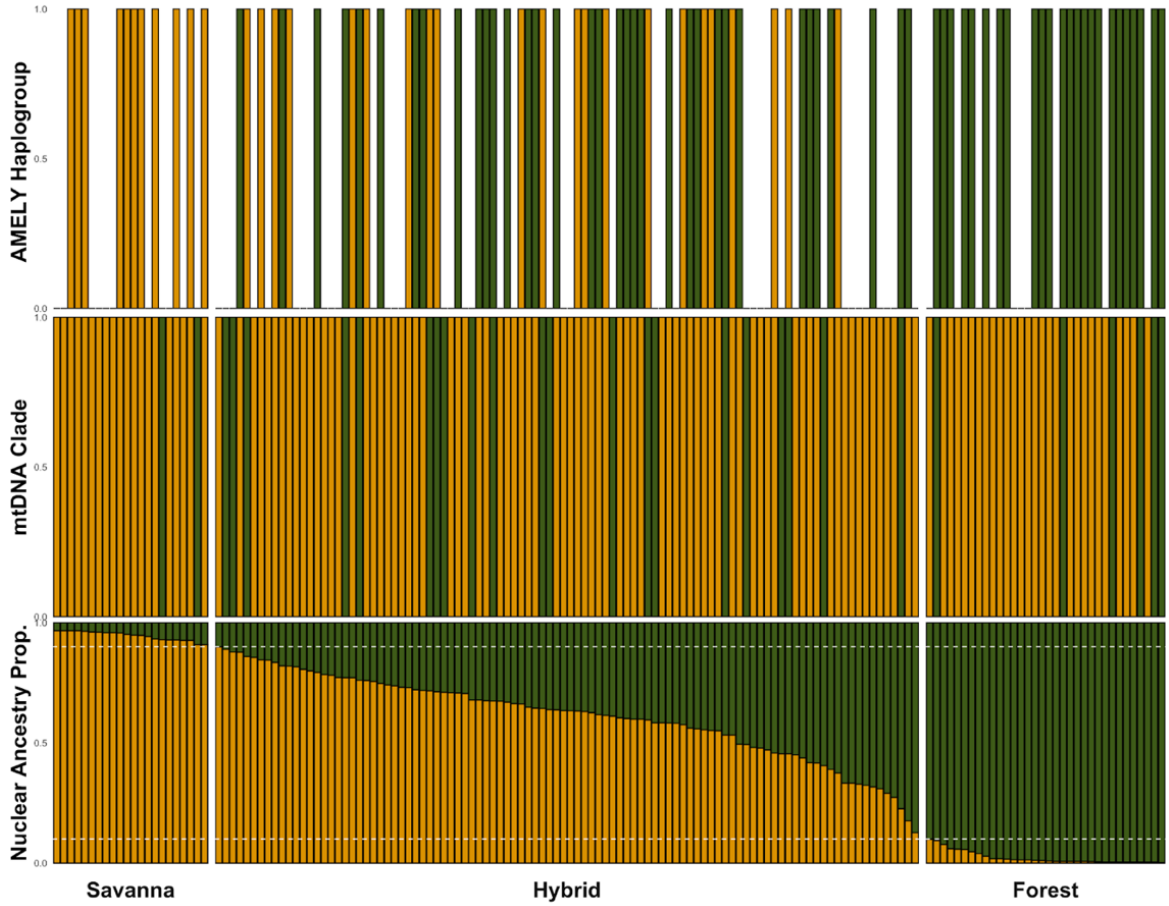


Figure 4. Plots showing biparental nuclear ancestry based on 14 microsatellite loci, the mtDNA clade, and the AMELY haplotype for each individual analyzed in this study demonstrate different distributions of genetic ancestry across these three differentially-inherited markers. Nuclear ancestry proportions were derived from STRUCTURE output at K=2. Each bar represents one unique individual, with yellow indicating savanna-typical ancestry, and green indicating forest-typical ancestry.

Discussion

Social Groups are Structured by Female Philopatry Rather than Species Identity

Our findings suggest that hybrid elephants living in a mixed-species population maintain some aspects of social structure consistent with their parental species (Archie et al., 2006; Schuttler et al., 2014b), and that matrilineal social organization does not break down in a hybrid population. This is despite evidence that African elephant matrilineal structure may break down in heavily human-altered environments (Brand et al., 2020) like Western Uganda. Our female network showed higher relatedness values within components than between components, indicating that associations in this population are structured along lines of relatedness. Furthermore, females showed a high degree of assortativity by mitochondrial haplotype and shared the same mitochondrial haplotype in 86% of the components which we detected. Males, in contrast, showed extremely low levels of assortativity by mitochondrial haplotype and did not have elevated relatedness within components relative to between components. These findings are highly consistent with the existing literature for both forest and savanna elephants. Female savanna elephants in Amboseli National Park are more related within than between components and share a uniform mitochondrial haplotype in 95% of core groups and 80% of bond groups (Archie et al., 2006). Similarly, female forest elephants in Lopé National Park are also more related within than between components, and share a uniform mitochondrial haplotype in 84.6% of groups (Schuttler et al., 2014a).

Taken together, the indirect genetic evidence from our work suggests that the hybrid elephant population of Western Uganda maintains a social structure consistent with female philopatry and male dispersal (Archie et al., 2006; Charif et al., 2005). Notably, our networks were also more strongly assortative by mitochondrial haplotype than by species identity. This

finding suggests that matrilineal sociality outweighs species identity in structuring mixed-species elephant societies, and that the matrilineal social structure of pure forest and savanna elephants does not break down in a hybrid zone.

Female Group Size is Correlated with Genetic Ancestry

Our results also suggest that genetic ancestry may play a role in behavioral differences between parental species. Female forest elephants typically associate with smaller core groups than female savanna elephants. However, because elephant behavior is highly flexible, it is unknown whether this trait is due to differing environmental constraints across the respective ranges of these two species (Gobush et al., 2021a, 2021b), or to underlying species-typical differences. The pure forest elephant components detected in this work were comparable in size (1.6 ± SD 0.9 females) to those described in the forest elephant population at Lopé National Park (1.48 ± SD 0.8 adult females), while the pure savanna elephant components were on average smaller (2.5 ± SD 2.1 females) than those of the savanna elephant population at Samburu National Park (5.03 ± SD 4.61 adult females). This latter result should be interpreted with caution, given that only two pure savanna elephant components were detected in our female network. Instead, most of the pure savanna elephants in this study were detected in mixed-species groups with hybrid individuals (6.3 ± SD 1.9 females).

To account for this constraint, we further tested for a correlation between the average genetic ancestry of a component and the size of that component in our female network. We found that female group size in the Western Ugandan hybrid elephant population is correlated with genetic ancestry: female network components with a higher average proportion of savanna elephant ancestry were more likely to be detected in association with a larger number of unique individuals. Although few pure parental components were found in this study, this finding

suggests that hybrid and mixed-species groups may also exhibit intermediate behavioral phenotypes wherein genetic background partially correlates with aspects of parental social structure. Intermediate behavioral phenotypes such as this have been demonstrated in hybrids across a diversity of taxa including primates (Bergman et al., 2008), birds (Pearson & Rohwer, 2000) and fish (Moore et al., 2010), and this study suggests that they may also be characteristic of hybrid elephants. Further work is needed to test whether additional behavioral differences between parental species (i.e., diet, communication, migration) are also intermediate in hybrids, and what the consequences of intermediate behavioral phenotypes might be for the conservation and management of these animals.

Notably, we also found that mixed-species components in our global network constituted the largest groups detected in our study and were on average larger than hybrid groups and the monospecific groups of both parental species. One explanation for this finding is that this result is a consequence of sampling probability, and that as the number of elephants detected at a location increases, so too does the likelihood of detecting at least one individual with a different genetic ancestry. Alternatively, this finding could indicate that more complex social processes – beyond a simple intermediate-phenotype explanation – may be at play in this hybrid population. For instance, although forest elephants primarily associate in small core groups consisting of a female and her dependent offspring, these small groups are known to join larger groups when they are visiting saline bais. It is thought that, because minerals at bais are not a limiting resource, these clearings serve as "social arenas" for otherwise more solitary individuals to exchange information and maximize social opportunity (Fishlock & Lee, 2013). It is therefore possible that, in certain contexts, elephants with different genetic ancestries might also associate in large, mixed-species groups to facilitate social exchange.

Forest Elephant Males Form Bachelor Herds

Little work to date has been done to examine aspects of sociality in forest elephant males. However, limited data suggest that males of this species are more likely to be solitary and less likely to form bachelor herds than their savanna elephant counterparts. One study of forest elephant males at Dzanga Bai in the Central African Republic (Turkalo et al., 2013) found that the majority of adult males were solitary when they entered the clearing, and that multi-male bachelor herds were rare (0.3% of detections). In contrast to this previous work, we found that forest elephant males in the Western Uganda population were no more likely to be found solitarily than were hybrid or savanna elephant males. 40% of the putatively pure forest males in this study were detected in all-male bachelor herds, and only 23% were detected solitarily. This finding is more comparable to previous work in savanna elephant populations, including in Amboseli where different studies have alternatively shown 63% (Chiyo et al., 2011) and 30.5% (Lee et al., 2011) of males to associate in multi-male bachelor herds, and only 12.5% to be solitary (Lee et al., 2011). Furthermore, in contrast to the negative correlation observed in the female network, we did not find any evidence for a correlation between genetic ancestry and bachelor herd size in our male network, suggesting that bachelor herd size does not vary by species type in this population. In savanna elephants, bachelor herds serve multiple functions including providing opportunities for young males to assess their relative positions within a dominance hierarchy and to learn from older males (Allen et al., 2020; Evans & Harris, 2008). No detailed study on forest elephant male sociality has been conducted to date, but our findings indicate that forest elephant males may be more social than current data suggest, and that further research is warranted to examine social structure and organization in males of this species.

Hybridization in Western Uganda is Context Dependent

We non-invasively interrogated characteristics of the mating system of the Western Uganda hybrid elephant population using uniparentally inherited genetic markers, and found that hybridization in this region is bidirectional, meaning that males and females of both species hybridize with each other. This result is consistent with a previous study which examined 15 hybrid males from across Uganda, Rwanda and the Central African Republic to assess the direction of hybridization at the continent scale (Mondol et al., 2015). Here, we build upon these findings by examining higher resolution, site-specific patterns of hybridization.

Hybridization in Kibale National Park is Ongoing and Bidirectional

Our results suggest that the elephant population of KNP is characterized by ongoing, bidirectional hybridization. We did not find evidence for sex-biased hybridization at this site; rather, 50% of the males in this park carried the savanna-typical Y chromosome, while the remaining 50% carried the forest-typical haplotype. All four combinations of uniparental markers were also detected in the hybrid males of KNP, indicating that hybridization events in this population involve mating between forest females and savanna males and between savanna females and forest males. Previous work has demonstrated that the wide distribution of hybrid indices which characterize the elephant population of KNP are consistent with the expectation of a hybrid swarm (*Chapter 2*; Figure S2.1). Our uniparental data build upon this finding by suggesting that the mating system of this hybrid population is not biased with respect to genetic ancestry. Hybrid swarms can result from either 1) a breakdown of reproductive isolation between two incipient species, or 2) introgression between allopatric species following secondary contact (Hasselman et al., 2014). While our findings cannot fully disentangle these two explanations for

this population, they do suggest that, in some sympatric contexts, the mating systems of forest and savanna elephants are not sufficient to maintain species boundaries in a contact zone.

Historical Hybridization in Bwindi Impenetrable National Park was Sex-Biased

In contrast to KNP, we found that the patterns of genetic ancestry represented by the elephants of BINP were consistent with historical, sex-biased hybridization. BINP harbors primarily forest elephants which exhibit a narrow, unimodal distribution of hybrid indices (*Chapter 2*; Figure S2.1). We found that 100% of the males at this site carried the expected forest elephant typical Y chromosome, but that the population also showed surprisingly high levels of cytonuclear discordance: 84.8% of putatively pure forest elephants carried mtDNA derived from the savanna elephant lineage, including 100% of the females. Cytonuclear discordance is a well-established phenomenon across the *Loxodonta* genus (Roca et al., 2005) and can be explained by the social systems of these species. Because females are philopatric and males disperse from their natal groups when they reach reproductive maturity (Archie et al., 2007; Nyakaana & Arctander, 1999), the maternally-inherited mitochondrial genome can inform upon more ancient phylogeographic patterns of elephant occupancy at a geographic location after the nuclear genome has been "homogenized" by gene flow from heterospecifics dispersing into a population (Roca et al., 2005; Toews & Brelsford, 2012). To date, introgression of congeneric mtDNA in elephants has largely been unidirectional: F-clade mtDNA has been detected in pure savanna elephant populations across eastern and southern Africa (Debruyne, 2005; Ishida et al., 2011, 2013; Roca et al., 2005, 2007). This pattern is consistent with multiple generations of forest (and subsequent hybrid) females crossing with savanna elephant males, and has been explained in terms of the competitive advantage that larger savanna elephant males would presumably have

over smaller forest elephants (Roca et al, 2005). Interestingly, however, we see the opposite pattern in BINP: our findings suggest that the elephants currently inhabiting BINP instead represent largely unidirectional, historical hybridization wherein forest males crossed to savanna females.

One possible explanation for this pattern could be that the elephants of BINP were historically a savanna-elephant population, but that forest elephant males migrated into the region and homogenized the nuclear genome through multiple generations of hybridization and backcrossing (Roca et al., 2005; Toews & Brelsford, 2012). At the landscape scale, the region that is now BINP was once connected to the Maramagambo Forest in what is now Queen Elizabeth National Park, a population to its north which is occupied by primarily savanna elephants (Plumptre et al., 2008; Taylor, 1992). However, the connection between these two areas was severed by landscape conversion to agriculture prior to 1929, and possibly as long as 2,200 years ago (Hamilton et al., 1986; Taylor, 1992; Taylor & Marchant, 1994). On a more recent timescale, the earliest available documentation of elephants in this region indicates that in 1929 there was a resident elephant population which seasonally migrated to the Congo (Brooks & Buss), but that BINP was effectively isolated from the greater landscape by the late 1950s due to land conversion around its boundaries (Brooks & Buss, 1962; Plumptre et al., 2007). It is therefore possible that the history of land use change in this region may have initially isolated a subset of the large savanna elephant population to its north within BINP for an extended timescale, while simultaneously allowing for the dispersal of forest elephant males from the Congo into the park up until the late 1950s.

This landscape-scale process could explain the origin of the forest elephant males in BINP, but it fails to explain why forest males would homogenize the population, assuming that

larger savanna elephant males also occupied the forest. We propose several possible explanations for this pattern. The first possibility is that, due to intense poaching in BINP in the late 1970s which reduced the elephants to a remnant population of 22 individuals (Babaasa, 1994), there have not been any savanna elephant males in this park in the last ~50 years. Because poaching pressure targets larger individuals (savanna elephants), it is possible that intensive selective poaching at this time removed all of the savanna elephant males from this population. This process could explain the observation that 100% of the males in BINP carry forest-typical Y chromosomes, but because the introgression of S-clade mtDNA into a fully forest-typical nuclear genetic background would take a minimum of 10 generations (~200-250 years) of unidirectional backcrossing to appear (Roca et al., 2005), it fails to fully explain the patterns which we observe. A second possibility is that intraspecific competition between males might play less of a role in an individual's fitness in a forested environment than it does in an open savanna environment, a hypothesis which has been suggested due to the less pronounced sexual dimorphism seen in forest elephants than their savanna counterparts (LaDue et al., 2021; Roca, 2019). The third proposed explanation is that forest elephant males may have outcompeted savanna males in this park, either due to direct competition, a greater ability to find mates in the forested environment, or a female preference for heterospecifics. Heterospecific preference has been documented in a number of systems, and could lead to the nuclear and uniparental patterns which we see in the park today (Wirtz, 1999). Ultimately, none of these hypotheses are testable within the scope of this study, and further work is needed to elucidate the history of admixture in this population. However, the genetic patterns that we do see suggest a complicated history of hybridization in this park which has occurred for at least 10 generations, and which could potentially be the result

of a number of interacting factors including female philopatry, natural and human-induced landscape changes over time, and recent poaching pressures.

Open Questions

This work represents the first characterization of sociality in a hybrid elephant population. Using non-invasively collected genetic data, we reveal that matrilineal social organization is maintained in this population despite the history of elephant poaching and habitat conversion in the region, but also find that hybrid elephants may exhibit certain aspects of social structure (i.e., group size) which are intermediate to their two parental species. We also find that mating in this hybrid population is bidirectional but heterogenous across both space and time. The conclusions of this study are limited by the non-invasive nature of this work. However, our results highlight a number of new questions which may direct future investigation into the effects of species identity on elephant sociality and hybridization.

First, while our work enabled insight into associations between unique individuals, the density of sampling in this study limited our ability to construct association indices for elephants in this hybrid population. This limitation leaves a number of questions about the relative strength of relationships between individuals, as well as the complexity of these social dynamics across time, unanswered. For example, it has been proposed that savanna elephants exhibit a more complex, multi-tiered social structure than do forest elephants (Schuttler et al., 2014b; Fishlock & Lee, 2013; Hedwig et al., 2021). While direct observational studies would be considered the gold standard to quantify complex fission-fusion dynamics in this population (Moss et al., 2011; Wittemyer, 2001; Turkalo et al., 2013), the densely forested habitat that characterizes KNP and BINP makes this method infeasible for this population (and for forest elephant populations more generally). We alternatively suggest that longitudinal, non-invasive sampling of the elephants of

the Western Ugandan hybrid zone across multiple years would enable insight into the relative stability of the associations detected within our networks over time and allow us to further disentangle the effects of species-identity on social complexity in this system.

Furthermore, while our work highlights patterns of association between elephants with different genetic ancestries, these data are entirely correlative, and we can only speculate about the functions of these associations based on what is currently known about the social systems of forest and savanna elephants. Two of our findings in particular highlight the need for further work to understand aspects of sociality across different elephant types. First, we found that forest elephant males were no more likely to be found solitarily, and equally as likely to be detected in bachelor herds, as hybrid and savanna elephant males. This finding emphasizes the current dearth of knowledge regarding the social lives of these males and encourages work to further characterize male social structure in forest elephants. Additionally, we found that mixed-species social groups were on average larger than all other detected components. This finding raises the question: is there a benefit to forming mixed-species social groups in hybrid populations? Large social groups in elephants have been proposed to both facilitate knowledge sharing (McComb et al., 2001; Moss et al., 2011) and provide protection from lions (Power et al., 2009; Joubert, 2006; Loveridge et al., 2006; Ruggiero, 1991). There are no lions present in either of the parks surveyed in this study, and so the risk of predation is likely not driving these large groups. However, it is possible that mixed-species associations could serve a function enabling information sharing among different individuals. Older individuals are thought to serve as "repositories of knowledge" about the landscape in elephant societies (McComb et al., 2001), and it is possible that mixed-species associations could serve a similar social function.

Finally, our uniparental sequencing results indicate that hybridization in this region exhibits context dependence in both space and time. This is an important finding for our understanding of the factors that can drive hybridization between elephant species, but different scenarios could also explain the distributions of uniparental genetic markers that we see today. For example, the high degree of cytonuclear discordance that we observed in BINP could be due to many alternative explanations, as detailed above. To disentangle these alternative explanations for the history of this hybrid population, genomic scale data collected from across Western Uganda would be necessary. Given the contrasting patterns of uniparental inheritance that we observed between BINP and KNP, sequencing hybrid elephants across multiple sites could further elucidate the variation in the history of hybridization throughout this region both at the landscape scale and throughout time.

CHAPTER IV

CONCLUSION

Elephant populations across much of the African continent face severe rates of decline due to poaching and habitat loss: although as many as 26 million elephants were once widely distributed across sub-Saharan Africa (Rosencranz & Sehgal, 2017; Groves et al., 2000), fewer than 500,000 African elephants remain in the wild today (CITES, 2022; Gobush et al., 2021a; Gobush et al., 2021b). Forest elephants are particularly imperiled, constituting only ~56,000 of these remaining elephants following an 86% decline over the past three generations (CITES, 2022; Gobush et al., 2021b). Yet due to the logistical constraints of observing elephants in densely forested environments, this species remains largely understudied and their management needs are not fully understood. The recent decision by the International Union for the Conservation of Nature (IUCN) to separately assess African forest and savanna elephants on the IUCN Red List better highlights the differing threats of extinction faced by these two species and encourages further work to characterize species-typical differences. However, it also leaves taxonomically undefined or admixed populations in a series of priority sites across the continent with an uncertain conservation status (Hart et al., 2021; Bauer et al., 2021). Throughout this dissertation, I elucidated the dynamics of hybridization for the elephants of two forested sites in Western Uganda, the region which harbors the largest known modern hybrid zone between forest and savanna elephants. My findings inform on the conservation of elephants throughout this region, more broadly examine the consequences of hybridization for these species, and provide evidence-based recommendations for the monitoring and management of other taxonomically undefined populations across the continent.

In Chapter II, I applied non-invasive genetic monitoring techniques to characterize the landscape-scale dynamics of the hybrid elephant population in Western Uganda. This work represents the first high-resolution assessment of abundance and species identity for the elephants living in the forested protected areas of this region. Using high throughput amplicon sequencing and fecal-DNA based Capture Mark Recapture analysis, I jointly inferred census population estimates and species distribution data for the elephants of Bwindi Impenetrable and Kibale National Parks. I demonstrated that distinctive, site-specific patterns of abundance and species identity characterize these parks: Bwindi Impenetrable National Park harbors a less dense elephant population that consists primarily of forest elephants, while Kibale National Park harbors a denser population of primarily hybrids. Currently, technical guidance with respect to the classification of hybrid elephants is lacking from major conservation and regulatory authorities (Bauer et al., 2021), but these findings suggest that landscape-scale factors can contribute to the patterns of hybridization and species identity that we observe throughout the region today and highlight that site-specific monitoring and management efforts will be critical to developing conservation policy for admixed elephant populations moving forward.

Internationally, there are currently two conservation endeavors underway for which this research is informative: 1) the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) is in the process of updating their regulations to reflect a split, two species listing status for African elephants, and 2) the next iteration of the African Elephant Status Report (last published in 2016: Thouless et al., 2016) is set to be released in the upcoming year, with separate reports for each species (CITES, 2022). Additionally, at the national scale, the results of this chapter emphasize that Uganda is a range state for both forest and savanna

elephants and should reflect this status as it develops the next iteration of the country's national elephant conservation management plan, which is currently set to expire in 2026 (UWA, 2016).

While this work is informative for national and international conservation authorities, the findings have both positive and negative implications for the status of the critically endangered forest elephant. I found that although Kibale National Park is a primarily forested site that hosts a large elephant population, very few (< 1%) of the elephants in this park are pure forest elephants; however, I also found that Bwindi Impenetrable National Park hosts a population of primarily forest elephants which is more than twice the current estimate on record for this park. Although the countries of Central Africa—most notably, Gabon and Republic of Congo—constitute the world's last remaining strongholds for forest elephants (CITES, 2022), severe poaching has severely reduced the forest elephant populations of these countries over the past two decades (Poulsen et al., 2017; Blake et al., 2007; Gobush et al., 2021b). The considerable protections in place for the animals of Bwindi Impenetrable National Park (Hickey et al., 2019) therefore provide a small but significant stronghold for this species in Western Uganda. Notably, the elephant population of Bwindi Impenetrable is also of high conservation value due to its location within the Greater Virunga Landscape, which overall hosts a large elephant population and is a globally important site for its high rates of endemism and biodiversity (Plumptre et al., 2007; Figure 2.1). Although no corridors currently exist between the park and this greater landscape, conservation action could be prioritized to restore historical corridors for the elephants of Bwindi Impenetrable and could enable this park to serve as a source population for forest elephants in the Greater Virunga Landscape more broadly. Finally, high amounts of genetic diversity characterize forest elephants across the African continent (Roca et al., 2001, 2005; Comstock et al., 2002; Rohland et al., 2010; Ishida et al., 2011; Palkopoulou et al. 2018), and the elephants of

Bwindi Impenetrable capture both nuclear (*Chapter 2*) and mitochondrial (*Chapter 3*) genetic variation that is differentiated from elephants in the strongholds of Central Africa. Thus, although comparatively small, the forest elephant population of Bwindi Impenetrable is well protected and potentially evolutionarily significant, making its numbers an important contribution to the remaining forest elephants continent-wide.

In Chapter III, I further built upon my previous results by characterizing the social system of a hybrid elephant population for the first time. I demonstrated that aspects of forest and savanna elephant social organization – notably, female philopatry and male dispersal – are maintained in the Western Uganda hybrid population, but that some aspects of social structure (i.e., group size) are in part correlated with genetic ancestry. This finding suggests that hybrid elephants might exhibit intermediate behavioral phenotypes to their parental species which may exacerbate conservation issues. For instance, if hybrid elephants exhibit intermediate migratory behavior (Moore et al., 2010; Delmore et al., 2014) or dietary preferences (Hayden et al., 2011; Grant & Grant, 1996), then their current habitats may be insufficient to meet their resource needs and may promote increased human-wildlife conflict. Overall, these findings highlight that further work examining the impacts of hybridization on the behavior, health and fitness of hybrid elephants will prove critical to informing management efforts for these animals moving forward.

Finally, I found that – like the contrasting patterns of species identity identified in Chapter II – the two parks in this study also exhibited contrasting patterns of uniparental inheritance indicative of different histories of hybridization at the two sites through time. All of the males identified in Bwindi Impenetrable National Park carried forest-typical Y chromosomes, in spite of the fact that the majority of these same animals carried savanna-typical mitochondrial DNA. This pattern suggests a sex-biased history of hybridization captured by the

elephants in this park, wherein forest elephant males asymmetrically crossed to savanna elephant females. However, the "pure" nuclear genomes of these same elephants suggest that this process took place at least 10 generations in the past (Roca et al., 2005). The elephants of Kibale National Park, however, showed a different signature of uniparental inheritance: all four combinations of uniparental markers were detected in the hybrids of this park. Along with the broad distribution of hybrid indices for these animals (*Chapter 2*), these data suggest that hybridization is ongoing in Kibale National Park and that the elephants of this site exhibit genetic signatures characteristic of a hybrid swarm (Allendorf et al., 2001).

These findings in particular raise a difficult question which must be asked about Uganda's elephants: should we be prioritizing the conservation of "pure" species over hybrids? Although the elephants of Bwindi Impenetrable National Park adhere well to the species-level classification scheme under which national and international conservation policy is currently written (Allendorf et al., 2001; Draper et al., 2021), my work does not rule out the possibility that the elephants of Kibale National Park represent the more "natural" state of the elephant population in this region, especially in light of recent genomic data which have suggested that interspecies hybridization was a recurrent feature of elephantid evolution (Palkopoulou et al., 2018). For instance, while the more "pure" elephant population of Bwindi Impenetrable has been isolated from the Greater Virunga Landscape for nearly a century, the elephants of Kibale National Park are believed to maintain connections to this greater landscape. The cytonuclear discordance which I detected in Bwindi Impenetrable National Park further indicates that hybridization did occur in this population in the past, in spite of its primarily "pure" forest identity in the present day.

Historically, hybrid swarms like Kibale have largely been considered of little conservation value due to the genetic swamping of parental genotypes in these populations (Allendorf et al., 2001). However, recent advances in sequencing technology have revealed that hybridization is much more pervasive in nature than previously believed (Payseur & Rieseberg, 2016; Taylor & Larson, 2019), suggesting that a more nuanced approach to managing admixed populations may be required (Fitzpatrick et al., 2015; Jackiw et al., 2015; Quilodrán et al., 2020). Because my findings suggest that landscape-scale processes play a role in patterning species-identity across Western Uganda, it is possible that strategically placed corridors or buffer zones between populations (or, alternatively, increased isolation of populations) could be used to modulate species identity in the various parks of this region in the long-term. For this reason, it is critical that further work: 1) interrogate whether the elevated level of hybridization observed throughout this region is natural or human-induced using whole genome sequencing; 2) assess the health and fitness of hybrids, to discern whether conservation authorities and managers should prioritize maintaining non-admixed populations for the long-term survival of this population; and 3) examine the ecological niche (i.e. diet, nutrient cycling, ecosystem engineering) that hybrid elephants fill, especially with regard to whether admixed elephants play a similar functional role to one or both of their keystone parental species. These three critical pieces of information are difficult to robustly ascertain, but will ultimately be necessary for making the best-informed management decisions for this population.

Throughout this dissertation, I demonstrated that hybridization between elephants in Western Uganda is heterogeneous across space and time. The contrasting patterns of species identity and hybrid history that I found between Bwindi Impenetrable and Kibale National Parks contribute more nuanced data to the ongoing discussion about what factors can and do drive

hybridization between elephant species. As poaching, habitat conversion, and a changing climate continue to impact the fine-scale contexts within which Africa's two elephant species interact, continued efforts to understand the effects of hybridization on the elephants of Western Uganda will be critical to continent-wide elephant conservation both today and in the future.

APPENDIX A

SUPPLEMENTARY INFORMATION FOR CHAPTER II

Supplemental Figures

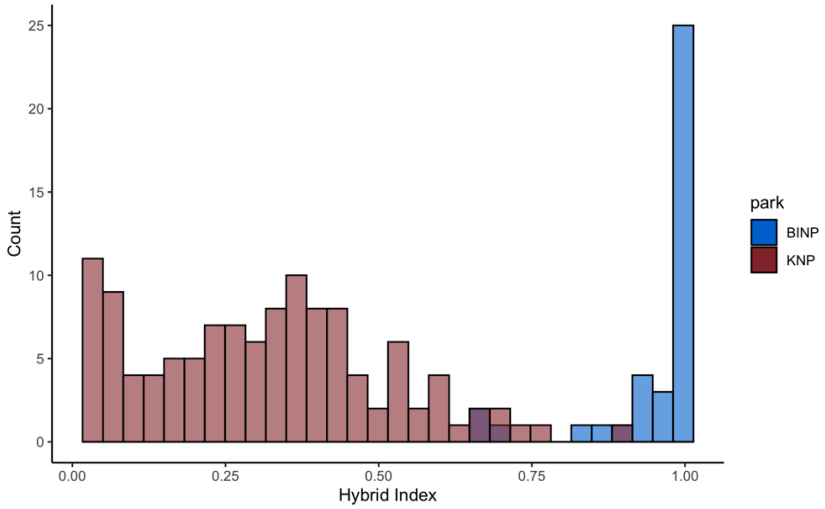
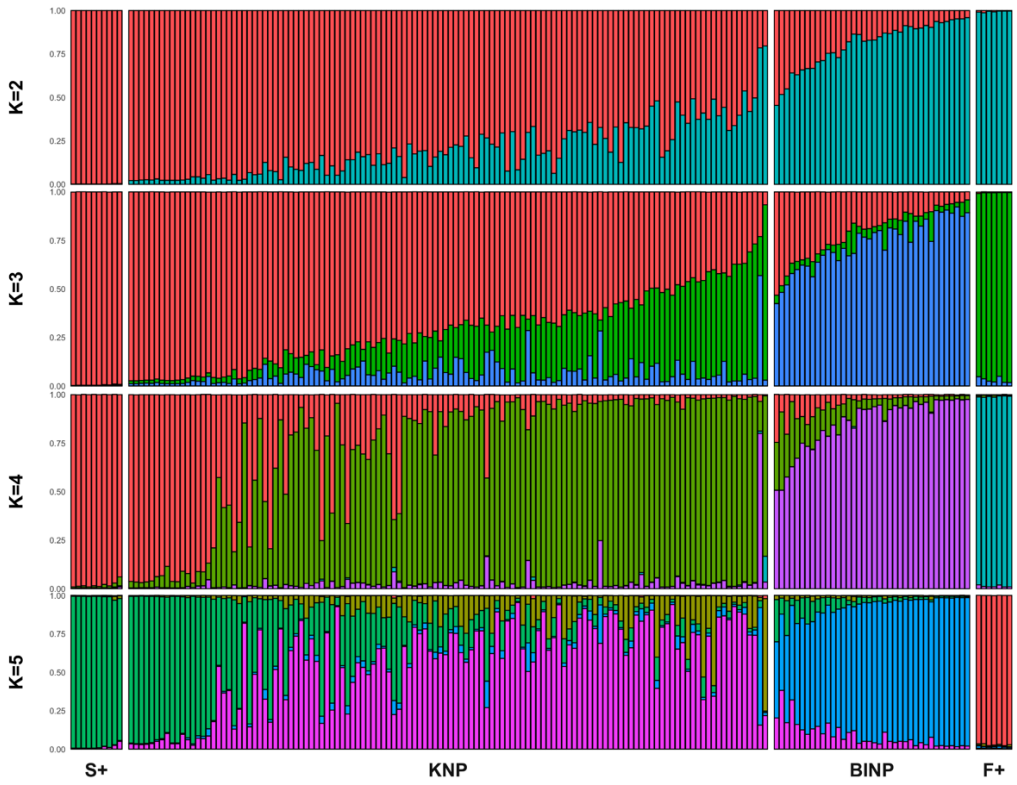
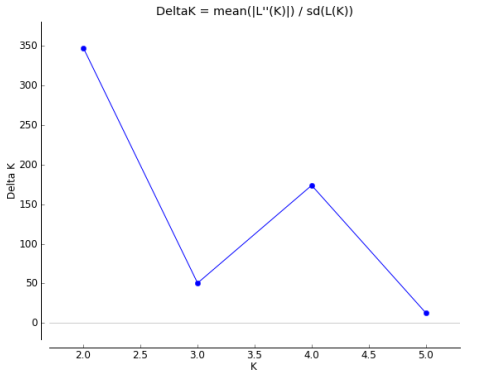
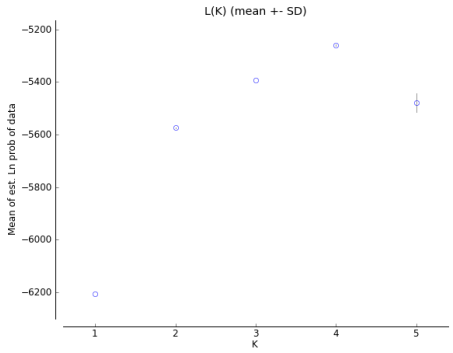


Figure S2.1. Distribution of hybrid indices for the elephants of KNP and BINP. A hybrid index of 0 denotes pure savanna ancestry, and a hybrid index of 1.0 denotes pure forest elephant ancestry. The elephants of KNP show a broad range of intermediate ancestries consistent with expectation under a hybrid swarm (Allendorf et al., 2001), while the elephants of BINP show a narrow, unimodal distribution around forest elephant ancestry.

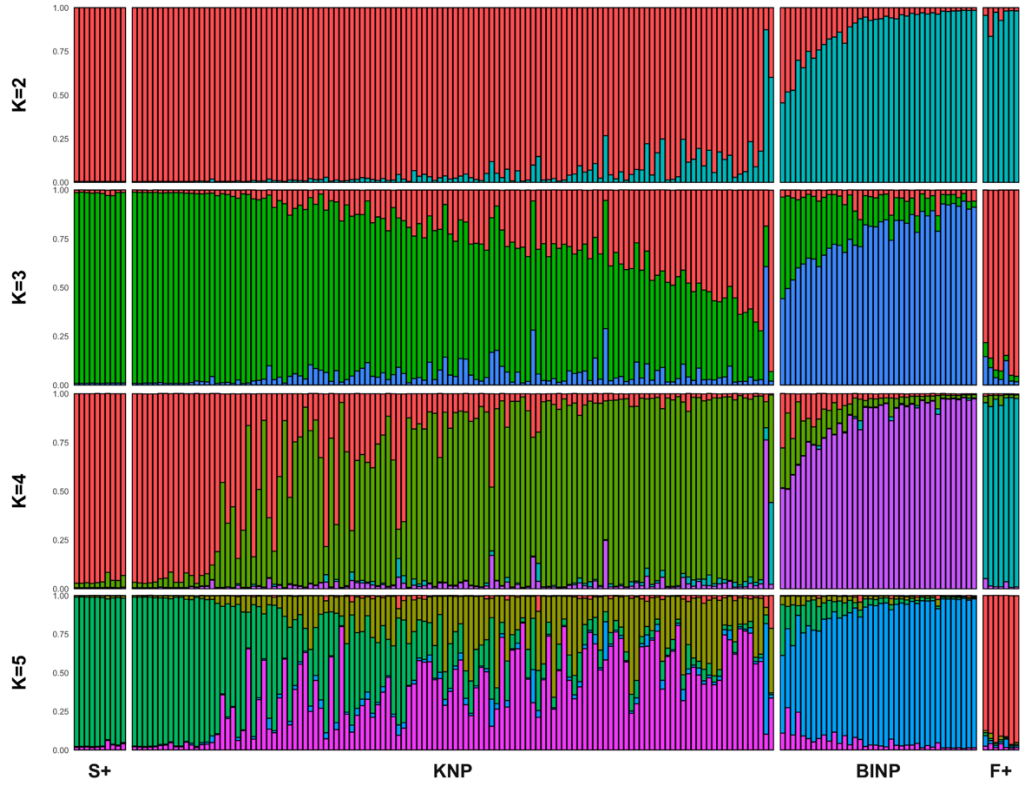


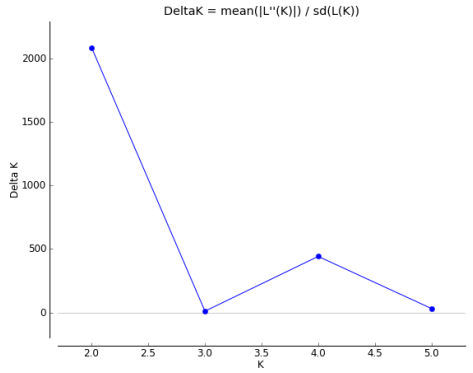


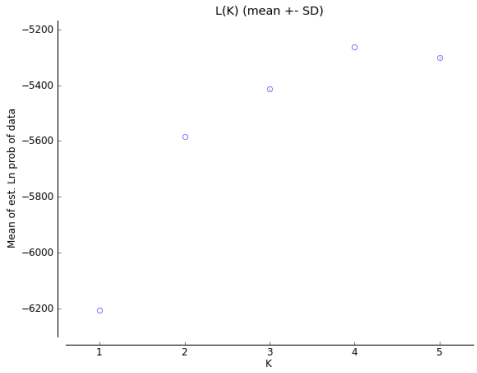


K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	5	-6206.040000	0.230217	—	—	—
2	5	-5574.260000	1.078425	631.780000	451.220000	418.406559
3	5	-5393.700000	0.924662	180.560000	46.240000	50.007457
4	5	-5259.380000	1.722498	134.320000	297.100000	172.482039
5	5	-5422.160000	59.876272	-162.780000	361.940000	6.044798

Figure S2.2. STRUCTURE HARVESTER results for the combined STRUCTURE run implemented with location priors for K=2 through K=5. Implementation of the Evano method indicates maximum support for population structure at K=2. Abbreviations above are: KNP = Kibale National Park; BINP=Bwindi Impenetrable National Park; F+ = Forest elephant positive control; S+ = Savanna elephant positive control.







K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	5	-6206.060000	0.270185	—	—	—
2	5	-5584.820000	0.258844	621.240000	449.480000	1736.492736
3	5	-5413.060000	1.957805	171.760000	20.960000	10.705868
4	5	-5262.260000	0.391152	150.800000	185.820000	475.058114
5	5	-5297.280000	4.399659	-35.020000	146.550000	33.309399

Figure S2.3. STRUCTURE HARVESTER results for the combined STRUCTURE run implemented without location priors for K=2 through K=5. Implementation of the Evano method indicates maximum support for population structure at K=2. Abbreviations above are: KNP = Kibale National Park; BNP=Bwindi Impenetrable National Park; F+ = Forest elephant positive control; S+ = Savanna elephant positive control.

Supplemental Tables

Sample ID	Species	Source
Lcyc01	*L. cyclotis*	Gamba Complex, Gabon
Lcyc02	*L. cyclotis*	Gamba Complex, Gabon
Lcyc03	*L. cyclotis*	Gamba Complex, Gabon
Lcyc04	*L. cyclotis*	Gamba Complex, Gabon
Lcyc05	*L. cyclotis*	Gamba Complex, Gabon
Lcyc06	*L. cyclotis*	Gamba Complex, Gabon
Lcyc07	*L. cyclotis*	Gamba Complex, Gabon
Lcyc08	*L. cyclotis*	Gamba Complex, Gabon
Lcyc09	*L. cyclotis*	Gamba Complex, Gabon
Lafr01 (SB208)	*L. africana*	Born: San Diego Wild Animal Park Dam: Zimbabwe; Sire: Zambia
Lafr02 (SB214)	*L. africana*	Born: San Diego Wild Animal Park Dam: Zimbabwe; Sire: Zambia
Lafr03 (SB76)	*L. africana*	Rhodes National Park, Zimbabwe
Lafr04 (SB114)	*L. africana*	Uganda (park unknown)
Lafr05 (SB527)	*L. africana*	Kruger National Park, South Africa
Lafr06 (SB533)	*L. africana*	Kruger National Park, South Africa
Lafr07 (SB532)	*L. africana*	Kruger National Park, South Africa
Lafr08 (SB528)	*L. africana*	Kruger National Park, South Africa
Lafr09 (SB531)	*L. africana*	Kruger National Park, South Africa
Lafr12 (SB540)	*L. africana*	Born: San Diego Wild Animal Park Dam: South Africa; Sire: Unknown

Table S2.1. Origins for positive control DNA used for species assignment analysis in this study. *L. cyclotis* samples (n=9) were collected across the Gamba Complex of Protected Areas in Gabon. *L. africana* samples (n=10) were provided by the San Diego Frozen Zoo (BRG#2020036).

Capture(s)	KNP	BINP
1X	104	28
2X	14	10
Total	132	48

Table S2.2. Capture rates for KNP and BINP. Numbers denote individuals recaptured in separate 30-day sampling bouts.

APPENDIX B

SUPPLEMENTARY INFORMATION FOR CHAPTER III

Supplemental Figures

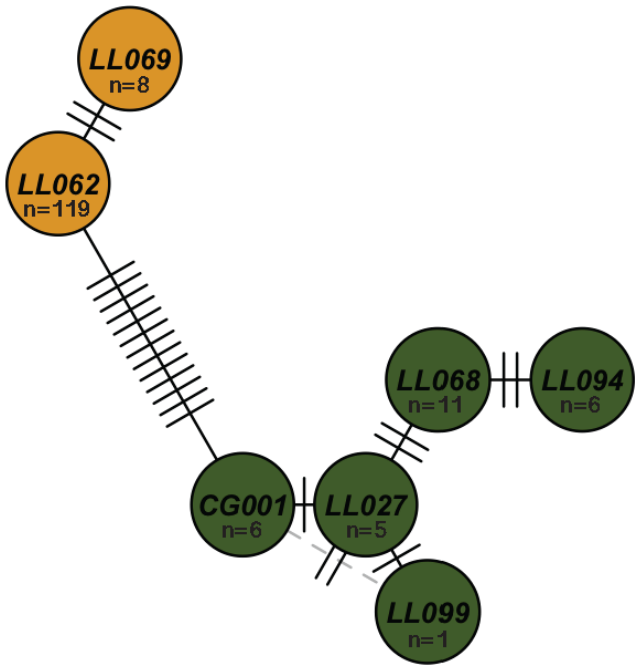
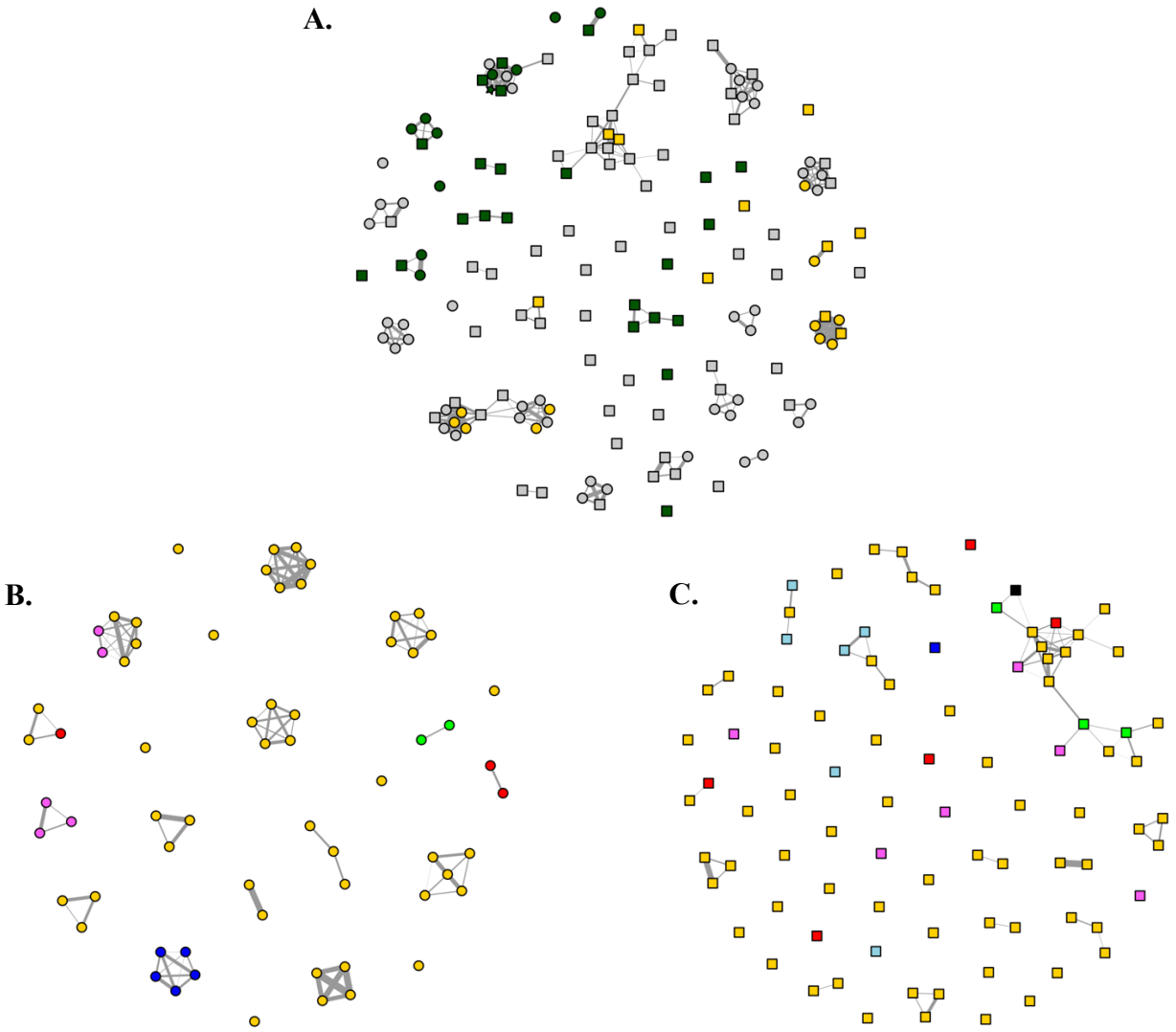


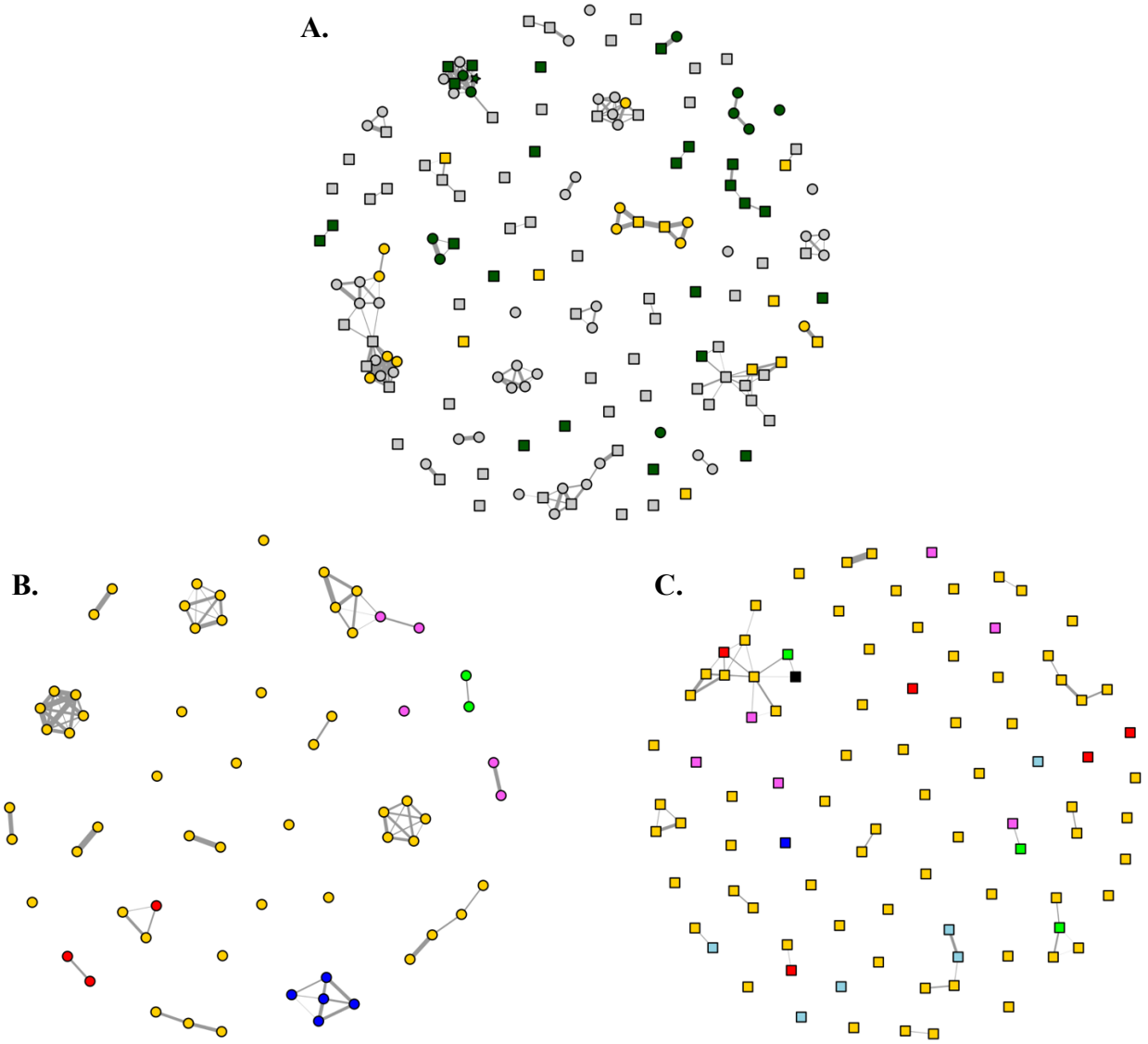
Figure S3.1. Haplotype diagram for the 319 bp segment of the mitochondrial control region sequenced in this study. In total, 81.4% of the mtDNA sequenced in this study belonged to the S-clade, and 18.6% to the F-clade. The S-clade haplotype LL062 was the most frequently detected haplotype and was found in 76.3% of sequenced individuals. Yellow nodes denote haplotypes which exhibit S-clade variation, and green nodes denote haplotypes which exhibit F-clade variation. Sample size indicates the number of individuals in this study in which each mitochondrial haplotype was detected.



D.

Network	N	Nodes	Edges	Singletons	Components	Largest Component	Mean Component Size	
							With Singletons	No Singletons
Global	57	156	251	33	24	18	2.7 SD 3.4	5.1 SD 4.2
Female	22	64	92	7	15	6	2.9 SD 1.8	3.8 SD 1.4
Male	52	91	62	38	14	18	1.8 SD 2.4	3.8 SD 4.2

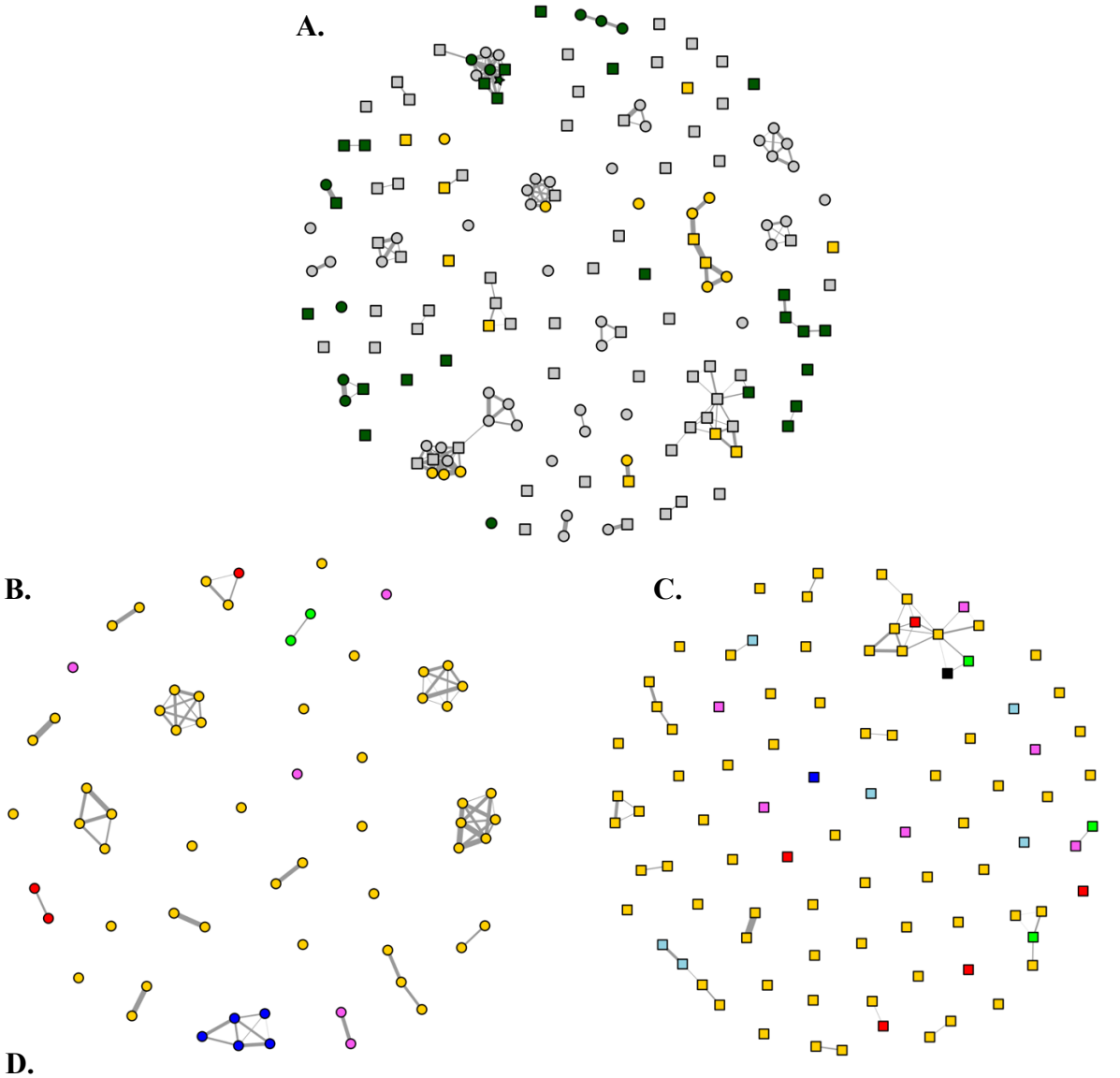
Figure S3.2. 250-meter networks. Nodes represent all unique individuals detected in this study, while edges represent associations detected using a 250-meter association distance. Node shape denotes sex (circle: female, square: male, star: unknown) and edge weights represent relatedness between individuals, with wider lines indicating more closely related individuals. A) Global network. Node color represents species classification based on 14 microsatellite loci (yellow=savanna, green=forest, gray=hybrid). B) Female network and C) Male network colored by mitochondrial haplotype. Node color represents unique mitochondrial haplotypes for 319 bp of the mitochondrial control region (Yellow: LL062, blue: CG001, pink: LL068, red: LL069, green: LL027). D) Descriptive statistics for 250-meter networks.



D.

Network	N	Nodes	Edges	Singletons	Components	Largest Component	Mean Component Size	
							With Singletons	No Singletons
Global	69	156	187	42	27	16	2.3 SD 2.7	4.2 SD 3.5
Female	27	64	69	11	16	6	2.4 SD 1.7	3.3 SD 1.6
Male	61	91	40	47	14	11	1.5 SD 1.4	3.1 SD 2.4

Figure S3.3. 100-meter networks. Nodes represent all unique individuals detected in this study, while edges represent associations detected using a 100-meter association distance. Node shape denotes sex (circle: female, square: male, star: unknown) and edge weights represent relatedness between individuals, with wider lines indicating more closely related individuals. A) Global network. Node color represents species classification based on 14 microsatellite loci (yellow=savanna, green=forest, gray=hybrid). B) Female network and C) Male network colored by mitochondrial haplotype. Node color represents unique mitochondrial haplotypes for 319 bp of the mitochondrial control region (Yellow: LL062, blue: CG001, pink: LL068, red: LL069, green: LL027). D) Descriptive statistics for 250-meter networks.



Network	N	Nodes	Edges	Singletons	Components	Largest Component	Mean Component Size	
							With Singletons	No Singletons
Global	78	156	163	51	27	13	2.0 SD 2.2	3.9 SD 3.0
Female	31	64	61	15	16	6	2.1 SD 1.5	3.1 SD 1.4
Male	62	91	39	48	14	11	1.5 SD 1.4	3.1 SD 2.4

Figure S3.4. 75-meter networks. Nodes represent all unique individuals detected in this study, while edges represent associations detected using a 75-meter association distance. Node shape denotes sex (circle: female, square: male, star: unknown) and edge weights represent relatedness between individuals, with wider lines indicating more closely related individuals. A) Global network. Node color represents species classification based on 14 microsatellite loci (yellow=savanna, green=forest, gray=hybrid). B) Female network and C) Male network colored by mitochondrial haplotype. Node color represents unique mitochondrial haplotypes for 319 bp of the mitochondrial control region (Yellow: LL062, blue: CG001, pink: LL068, red: LL069, green: LL027). D) Descriptive statistics for 250-meter networks.

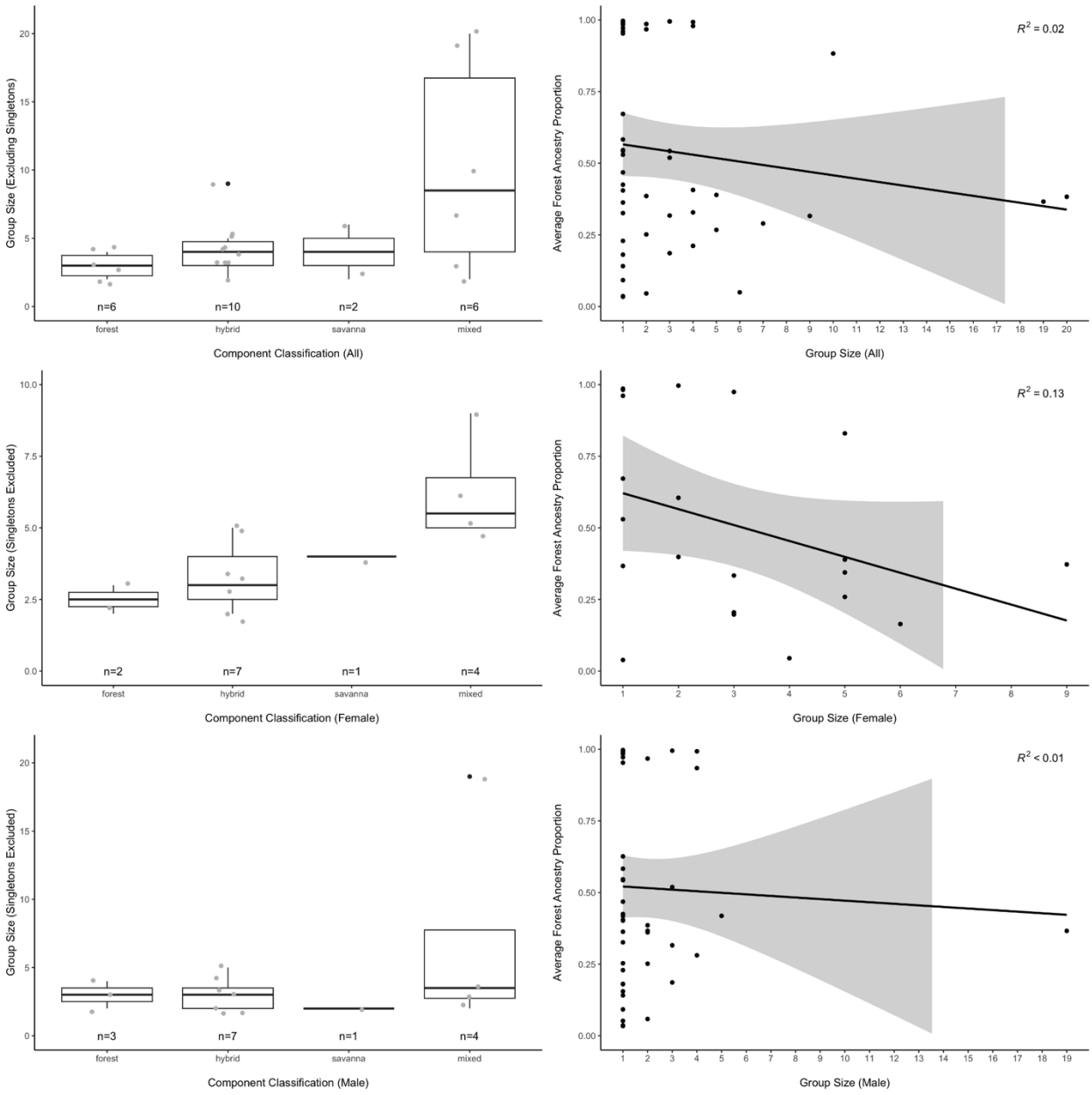


Figure S3.5. Group size and genetic ancestry plots for the three networks in this study, without excluding outliers. Component type vs. component size for the three networks in this study are presented in the left panel, while plots of group size vs. forest ancestry proportion derived from STRUCTURE at K=2 are presented in the right panel.

Supplemental Tables

Haplotype	Clade	Detections: Uganda (Protected Areas)	Detections: Continent-Wide
LL027	**F-clade**	**Kibale NP **** [1] Kidepo Valley NP [1] Murchison Falls NP [1] Queen Elizabeth NP [2] Semuliki NP [2]	DRC [3] Kenya [1] Tanzania [5]
LL062	**S-clade**	**Bwindi Impenetrable NP *,[2]** **Kibale NP *,[2]** Queen Elizabeth NP [1,2]	Botswana [5] Eritrea [6] Kenya [1,5,9] Namibia [5,7] Rwanda [2] South Africa [4,5] Tanzania [5] Zimbabwe [5,7,8]
LL068	**F-clade**	**Kibale NP **** [1] Queen Elizabeth NP [1,2] Semuliki NP [2]	DRC [2,5] Kenya [4] Rwanda [2] Zambia [5]
LL069	**S-clade**	**Kibale NP **** [1] Kidepo Valley NP [1] Murchison Falls NP [1,2] Queen Elizabeth NP [1]	Cameroon [5] Kenya [1,5]
LL094	**F-clade**	**Bwindi Impenetrable NP *,[2]** **Kibale NP ****	Rwanda [2]
LL099	**F-clade**	**Kibale NP **** [1] Semuliki NP [2]	*No detections*
CG001	**F-clade**	**Kibale NP **** [1]	*No detections*

Table S3.1. Geographic distribution data for the mitochondrial haplotypes detected in this study. Seven mitochondrial haplotypes were detected across 156 individuals. Six haplotypes have been previously detected in Uganda, although five haplotypes detected in this study represent the first detection of that haplotype in the park from which it was sampled. One haplotype (CG001) is not currently represented in public databases. [1.] Nyakaana et al., 2002; [2.] Mondol et al., 2015; [3.] Debruyne et al., 2003; [4.] Eggert et al., 2002; [5.] Ishida et al, 2013; [6.] Brandt et al, 2014; [7.] Debruyne et al., 2005; [8.] Charif et al., 2005; [9.] Archie et al., 2006 ***This study**; ****This study only, for submission to NCBI and *Loxodonta Localizer* databases.**

Network	N	Nodes	Edges	Singletons	Components	Largest Component	Mean Component Size		Edge Density	Mean Degree	Mean Path	Clustering Coefficient
							With Singletons	No Singletons				
BINP												
Global	17	38	54	10	7	10	2.2 SD 2.3	4.0 SD 2.8	0.04	2.84	1.17	0.96
Female	7	14	14	4	3	5	2.0 SD 1.5	3.3 SD 1.5	0.08	2.00	1.00	1.00
Male	15	23	10	11	4	4	1.5 SD 1.0	3.0 SD 0.8	0.02	0.87	1.23	0.67
KNP												
Global	34	118	220	17	17	20	3.5 SD 4.5	6.0 SD 5.4	0.02	3.73	2.03	0.87
Female	14	50	81	3	11	9	3.6 SD 2.3	4.3 SD 2.1	0.03	3.24	1.27	0.97
Male	34	68	65	23	11	19	2.0 SD 3.1	4.1 SD 5.0	0.01	1.91	1.98	0.73

Table S3.2. Descriptive social network statistics for each park (BINP=Bwindi Impenetrable National Park; KNP=Kibale National Park). Statistics computed in igraph.

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CHAPTER III

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CHAPTER IV

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