2006

Conservation genetics of wild born bonobos (Pan paniscus) using mitochondrial DNA

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CONSERVATION GENETICS OF WILD BORN BONOBOS (*PAN PANISCUS*) USING MITOCHONDRIAL DNA

A Thesis

Presented to

The Department of Biological Sciences

San Jose State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science

By

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December 2006

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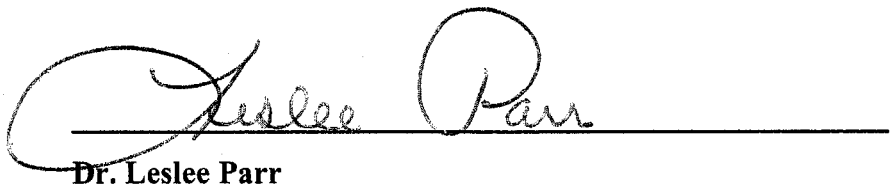

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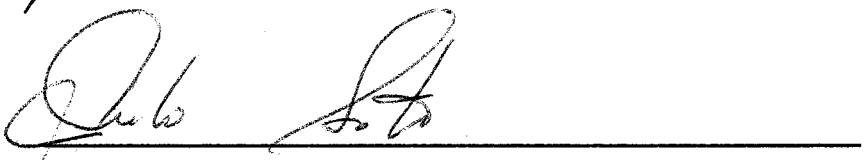

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ABSTRACT

CONSERVATION GENETICS OF WILD BORN BONOBOS (*PAN PANISCUS*) USING MITOCHONDRIAL DNA

by Amy E. Fontarensky

Mitochondrial DNA analysis of the HV1 region was performed on the orphaned bonobos, *Pan paniscus*, from Les Amis des Bonobos du Congo Bonobo (ABC) Sanctuary in Kinshasa, Democratic Republic of Congo. The results of this study were compared to published sequences of wild populations in order to determine the expected viability of the ABC bonobos as a founding population in their impending re-introduction to the wild. A subpopulation of ABC bonobos, with reported western origins, provides a model of the genetic make-up of bonobos from the western region.

ABC bonobo HV1 genetic diversity (H=0.890; π=0.30) was comparable to the genetic diversity of wild populations (H=0.781-0.923; π=0.023-0.038) (Eriksson et al. 2004); indicating no initial concern about potential inbreeding or outbreeding depression as a founding population. With the analysis of the western subpopulation, this study developed a recommendation for the preservation of a migration corridor between the wild central and western populations.

Dedication

This thesis is dedicated to my daughters, Katia and Natasha.

Katia and Natasha, all of what it took to achieve this goal was driven by my desire to set an example for you. Each time I saw how proud you were to have a mother who was a scientist, it made me want to be the very best I could be. All of the work that went into this achievement is dedicated to you.

Acknowledgements

I have been fortunate to be the deliverer of this work. I would not have been able to produce this thesis without a very large team of supporters, each with unique and major contributions that made it even conceivable.

Dr. Parr, I will never forget the day I walked into your office, with what I thought was a great, but overly optimistic idea, and you said, "Let's start tonight!" I had not even dared to believe that I could do genetic work –me, actually looking at the molecules of evolution, and being the first person to see them from this population of little known bonobos! On top of that, we were able to include applications to conservation. You said it was possible, you respected my family's needs, and you helped pave the way with encouragement, along with technical and financial support. I still cannot think of a more exciting project, apart from actually going out to collect samples from within the heart of Congo (which you still haven't discouraged)! You have altered the foundation of who I am, providing me with newly found confidence and a direction for guiding my daughters and students. Thank you!

Pierre, this whole project had the highest impact on you, who shared me with my passion for many years. You provided time, finances, encouragement, and household organization so that I could dedicate myself to this purpose. Also, without your continuous software support, I would not have been able to do any of the analysis required for this project. I share its success with you.

Mom, my pursuit of higher education would not have begun if it hadn't been for your financial and emotional support that came through great personal sacrifice on your part. You are the original source of this whole endeavor. My master's degree would not have happened without that unforgettable, business-like meeting that we had to discuss the prospects. Your support never wavered as you continued to travel miles and miles to help take care of Katia and Natasha, in order to provide me with time for my thesis. I hope I can provide such sustenance for Katia and Natasha so that they can achieve their dreams. Thank you.

Dr. Soto, Dr. Matson, and Chris Flynn, I am very grateful for your input in the form of critique and editing of my thesis. You each had pertinent and unique suggestions that contributed to the refinement of this project, which I aspired to take to the highest possible level. I appreciate each and every amelioration that you proposed.

I am very fortunate to have such supportive family and friends, with so many contributors to the completion of this project: both emotionally and through hours of dedication to my personal needs (usually childcare). Mom and Chris; Anna and David; Yolande and Serge; Aunt Helen and Uncle Steve; Dad and Lorna; and Valerie and David; thank you for forgiving my often neglect and delayed phone calls. All that you

have done, especially the love you have provided, has meant so much to my ability to accomplish what I consider a grand endeavor. With my deepest gratitude, Thank you!

Dr. Jurmain, you were the first one to trust me as a scientist, when you supported my design of a special project on chimpanzee behavior. It was in observing human's closest relative, at the Oakland Zoo, that I committed to my passion for evolution. Anne Warner, you educated me on the critical situation of the bushmeat trade in Africa. I realized through your resourcefulness that the priority of study for nonhuman primates should pertain to conservation. You introduced me to leading ape researchers, including Claudine Andre, who became the primary source of this project.

Claudine Andre, your dedication to bonobos is inspiring for all of us. Thank you for providing the circumstances that allowed this project to happen. Crispin Mahamba, it has been a pleasure collaborating with you. Thank you for providing samples and information in such a professional and timely manner. I hope we can work together again in the future.

In addition, I thank San Jose State University, ABC Bonobo Sanctuary, and the Oakland Zoo for their support in this project.

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INTRODUCTION

Relative to our understanding of other large bodied apes, little is known about the natural history and genetic make-up of bonobos (*Pan paniscus*). This study provides an analysis of a segment of the D-Loop of the mitochondrial DNA of a population of captive wild-born bonobos. The results are used to evaluate whether this population would make a viable founding population for re-introduction into the wild. The results are also compared to previously published studies of wild populations to further our understanding of the genetic composition and evolutionary past of the wild populations.

Bonobos are classified in the same genus as chimpanzees (*Pan troglodytes*), with which they are considered a sister species. It is estimated that bonobo ancestors were reproductively isolated from chimpanzee ancestors approximately 1.5 –2 million years ago, when the Congo River formed a barrier to gene flow (Thompson 1997). Bonobo ancestors resided in an isolated range, south of the Congo River, while chimpanzees colonized central Africa, north of the Congo River, between Tanzania and the Senegal (Teleki 1989).

Bonobos are endemic to the Democratic Republic of Congo (DRC). Because of the dense rain forest and the political instability that characterize their habitat, accurately assessing their demographics and population size has been problematic. With the compilation of small site reports, it was conservatively estimated that the bonobo population is between 20,000-50,000 individuals (Butynski 2001). This is based on information gathered since 1980 that did not take into account the period between 1998 and 2001, when DRC had been torn by extreme political instability (Butynski 2001).

Intense pressure from the commercial bushmeat trade (described below) is believed to have reduced the bonobo population dramatically in recent years. Bonobos are listed in Appendix 1 of the Convention on International Trade of Endangered Species (CITES) and classified as endangered in the International Union for the Conservation of Nature and Natural Resources (IUCN) Red Data Book.

Biogeographic History of Bonobos

Bonobos do not swim, and rivers are major barriers to migration and expansion (Kano 1992, Thompson 1997). Over the last 2 million years bonobos and chimpanzees have taken separate evolutionary routes. Bonobo ancestors resided essentially on an island between the Congo River and the Sankuru/ Kasai Rivers, each 2-15 kilometers wide (Beadle 1981) (Figure 1). In the middle proportion of its expanse, the Congo river flows slowly, forming a lacustrine-like environment, possibly similar to the lake that was located in the Congo Basin 7 million years ago (Beadle 1981). Bonobo evolution was shaped by periodic changes in the environment (Table 1).

Figure 1. Map of the Democratic Republic of Congo. Dotted line represents estimated range of bonobos.

Table 1. Outline of Bonobo Evolution Parallel to Congo Environment. Events are based on Hamilton and Taylor (1991) and Thompson (1997).

Approximate Time (millions of years ago)	**Congo Environment**	**Bonobo Evolution**
7 mya	Warm and Wet Tropical forests, inland sea	Common ancestor (CA) with Humans and chimpanzees (*Pan troglodytes)*
5 mya	Drying, Expansion Savanna Tectonic uplift east ➔Marunga Mountains	CA with Genera *Pan* and Proto-Homo split.
2.5 mya	Major glaciation Major temperature ↓; ↑ drying Inland sea drains.	CA with chimpanzees retreats tropical refuges.
2-1.5 mya	Interglacial Wet climate Congo River forms barrier	CA with chimpanzee reproductively isolated from chimpanzees
1.5-1 mya	Mostly glacial Dry climate	CA with chimps evolved into bonobo ancestors
1m-20kya	21 glacial/ non-glacial cycles; forest expansion and retreat	Bonobo ancestors migrate with forest expansion and retreat
20-12 kya	Glacial Maximum; Major forest reduction to remaining water sources	Bonobos retreat to refuges near water sources (Figure 2)
12k-100ya	Warming; Forest expansion	Bonobos migrated to current distribution
Present	Human logging, and agriculture reduce forest + hunting	Human induced reduction bonobo population

Gene flow between different populations of bonobo ancestors was significant enough to establish the species specific traits of present day bonobos, but has been relatively recently restricted by various river barriers, human hunting pressures, and habitat loss (Thompson 1997). If during the inter-pluvial maximum of Upper-Pleistocene, as hypothesized by Colyn (1987) and Colyn et al. (1991), tropical fauna followed the fluvial retreat patterns of the forests, then the various isolated bonobo populations likely experienced remote population bottlenecks. The founders of these isolated small populations are the ancestors of the current bonobo populations that remain secluded by major river barriers and large areas lacking bonobo distribution.

Over the last 2 million years, the climate in Africa went through several fluctuations in temperature and humidity. This created cycles of expansion and retraction of tropical forests (Hamilton and Taylor 1991). Bonobos are believed to be relatively restricted to tropical forest habitat, though it was discovered that bonobos are able to do some foraging in open savannah (Thompson 1997). With the climate changes bonobos would likely have had to follow the tropical forests. A model of the retreat of tropical forests at the glacial maximum, 20-12 thousand years ago, was developed (Colyn 1987, Colyn et al. 1991) (Figure 2).

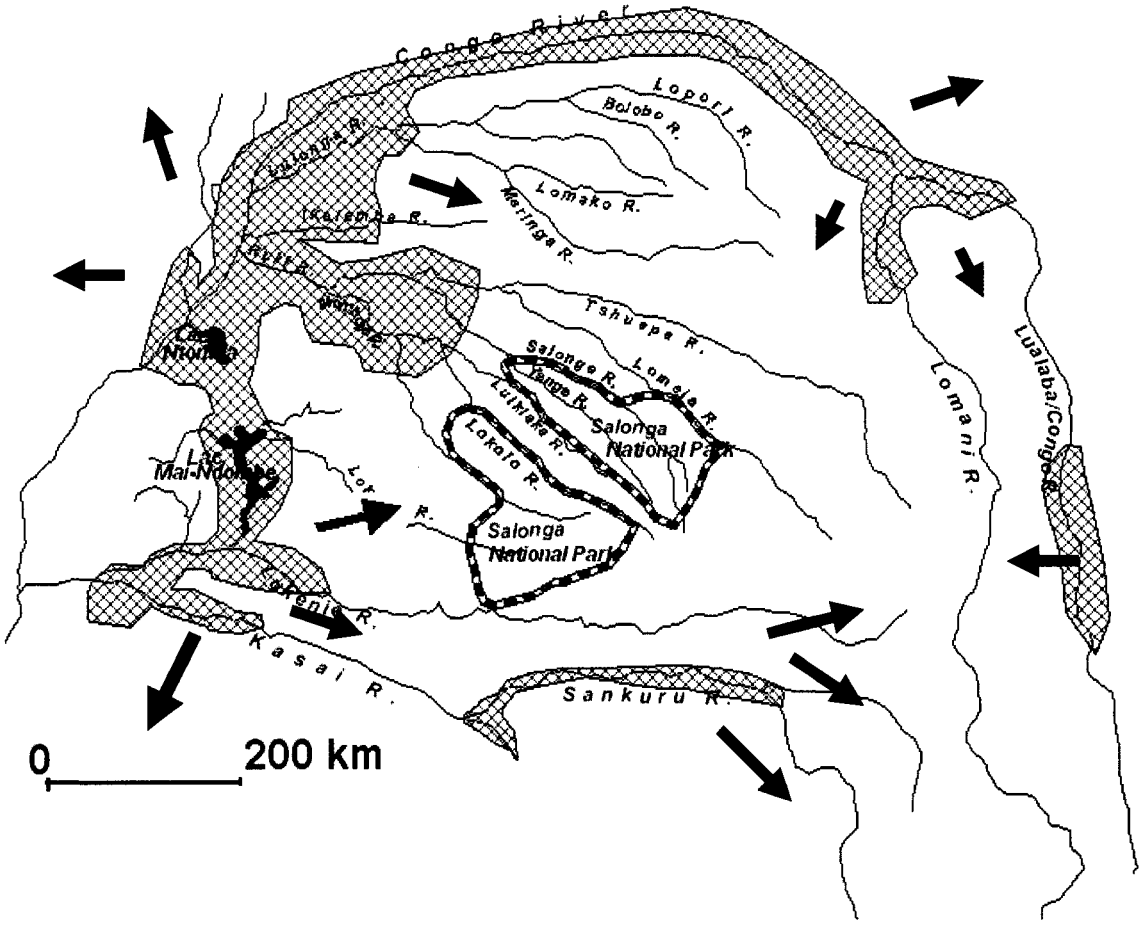

Figure 2. Hypothetical Ancient Migration Routes of Tropical Forest Fauna in the Congo Basin. Map is based on Colyn (1987) and Colyn et al. (1991) hypothesizing routes after the glacial maximum 12 thousand years ago, adapted for explaining bonobo distribution. The shaded regions represent the hypothetical forests along the permanent water routes. The arrows represent probable migration routes of tropical forest fauna from their refuges with the re-expansion of the forests.

Bonobo Life History

Geographic Distribution

The overall range of wild bonobos is estimated to be 500,000 km² (Thompson et al. 2003), distributed between the Congo/Lualaba River, forming eastern, northern, and western borders and the Sankuru/ Kasai River, bordering the south (Figure 1). Bonobos have been observed to inhabit dry forests, swamp forests, and secondary forests. Kano (1992) observed that the bonobos of the Wamba Forest obtain the majority of their food from dry forest. Bonobos from Lukuru do a substantial amount of foraging in open savannah, but are still reliant on adjacent forest for most foraging, and for nesting at night (Thompson 1997, 2001a).

The "minimum viable surface area" for a population of bonobos was estimated to be 300-600 km². A minimum viable population would consist of 300 individuals, divided into 5 groups, with 60 members each. This is calculated using the average density of individuals as 0.5-1.0 km (Thompson et al. 2003).

Social and Reproductive Structure

Like chimpanzees, bonobo social organization is classified as fission fusion. Bonobos aggregate in both small parties: between 4 and 17 individuals and larger communities, depending on time of the day, season, and activity (Hohmann and Fruth 2002). Relative to chimpanzees (Boesch and Boesch-Achermann 2000, Goodall 1986), available evidence indicates that bonobos have more peaceful interactions when encountering neighboring communities (Hohmann and Fruth 2002).

The mother-offspring unit is a significant grouping in bonobo society. It comprises the mother, her infant, her dependent juveniles, and her adult sons. Male bonobos are philopatric and stay in close contact with their mothers throughout their lifespan (Kano 1992).

Female bonobos migrate to other groups at the onset of sexual cycling at about age seven to nine. During the period of females' sexual sterility (the time between when they are cycling, and when they can actually conceive), she may change communities a few times. Females become permanent members of a community once they have given birth in that community, at about 13 years old. They have an estimated inter-birth interval of 4.5 years, with an approximated lifespan of 43 years (Thompson 1997, Kano 1992, Furuichi 1989).

History of Bonobo Research Correlated with DRC History

The history of intermittent research on wild bonobos has been tightly linked with the unstable political ambiance of the country. Unfortunately, DRC has a deep history of political instability (Edgerton 2002, Hochschild 1999), which has led to extreme poverty with the resulting lack of healthcare, education, and infrastructure. Recent dire humanitarian circumstances have not fostered conservation as a national priority, and have often hindered scientific research.

The fate of wild bonobos lies in the priority setting and decision-making of a single nation, the Democratic Republic of Congo (DRC). Bonobos are one of many endemic species in DRC, a country extremely rich in both biological and mineral

resources. Its main exports include diamonds, copper, coffee, cobalt, and crude oil. DRC covers 2.34 million square kilometers in equatorial Africa and has a population of 56 million people. It hosts 10,000 plant species, 400 fish species, 80 amphibian species, 1086 bird species, and 409 mammal species (Sayer 1992).

About 52.5% of DRC is forested (Sayer 1992). Seven national parks make up 82,600 km^2 and about 124,889 km^2 is dedicated to 22 reserves, together totaling 207, 490 km^2, or 9% of the national territory (Inogwabini et al. 2005). In the early 1990's, the government under President Mobutu, proposed to increase the amount of protected territory to 15% (Sayer 1992); however, the current politically defined reserves are mostly unprotected (Inogwabini et al. 2005) and DRC is now under the leadership of a different president.

Before European powers divided Africa into colonially defined territories, distinct languages and cultural traditions of over 1000 different ethnic groups determined political boundaries. With a relatively low human population size guided by traditional wisdom, hunting and agricultural practices remained sustainable. European decree placed borders that cut through traditional tribal territorial establishments and kingdoms and that forced traditional enemies to abide by the imposed leadership of the colonizing nation. This is a major source of today's political instability throughout Africa (Naughton-Treves and Weber 2001).

By 1885, after centuries of slave trade with the Kingdom of Kongo, King Leopold of Belguim officially claimed Congo. King Leopold ruled it under his exclusive and covertly brutal authority, calling it "Congo Free State." After Leopold's humanitarian

atrocities (including the continuation of slavery) were exposed by missionaries, Belgium annexed Congo in 1908 (Edgerton 2002, Hochschild 1999).

In the late 19th century, during Leopold's reign, European explorers started encountering specimens of apes that they could not clearly identify as either of the two contemporarily recognized species of chimpanzees (at that time, *Troglodytes schweinfuthii*, the eastern type, or *Troglodytes niger*, the western type). Various naturalists attempted to describe the type and distribution of this strange ape, which was given the name *Troglodytes schweinfurthi marungensis* and was considered a subspecies of chimpanzees. In 1929, Schwarz called bonobos *Pan satyrus paniscus*, a new subspecies taxon. In 1933, Coolidge, from the American Museum of Natural History, compared skulls of bonobos to those of chimpanzees and designated bonobos with the species status that they maintain today: *Pan paniscus* (reviewed by Thompson 1997, Thompson 2001c).

Between the 1930's and 1960's, a period characterized by much international strife, the international community lost interest in field research on apes. In the 1960's, extensive fieldwork began with the other species of African large-bodied apes, chimpanzees and gorillas, but that decade was a time of extreme political upheaval in DRC. Researchers didn't even know if bonobos had become extinct (Kano 1992).

In 1960, Congo gained independence from its 90-year colonial ruler, Belgium. Following independence there ensued a 40-year struggle for power, characterized by divided tribal loyalties, and fueled by the extremely high stakes of national riches. From 1965-1997, Joseph Mobutu ruled in DRC, re-naming it Zaire, letting the Belgium-

installed infrastructure fall to ruins, and driving the country into international debt (Edgerton 2002, Hochschild 1999). He did allow access to researchers, and was enthusiastic about conservation efforts. A network of national parks was established under his leadership, including Salonga National Park, which protects an area in the central part of bonobo range (Sayer 1992).

Research sites were established in the late 20th century (Figure 3 and Table 2). In 1972, Toshisada Nishida of Japan, gained access to Zaire and did a preliminary survey of the region west of Lac Tumba, confirming that bonobos were indeed present (Kano 1992). In 1973, Takayoshi Kano surveyed potential bonobo range in order to select a site for long-term research. He set up two sites in the northern part of the bonobo distribution, Yalosidi and Wamba (Figure 3). Also in 1973, Noel Badrian and his wife established another site, Lomako, in the north, about 150 km from Wamba (Kano 1992).

At about the same time two research units arrived from America. Yerkes Primate Center captured bonobos and took them back to their research center in order to breed a population for captive research. Yale University's Arthur Horn tried to follow up on Nishidi's report of bonobos west of Lake Tumba, but was not successful with bonobos, due to their low population density. He resorted to studying local monkeys (Kano 1992).

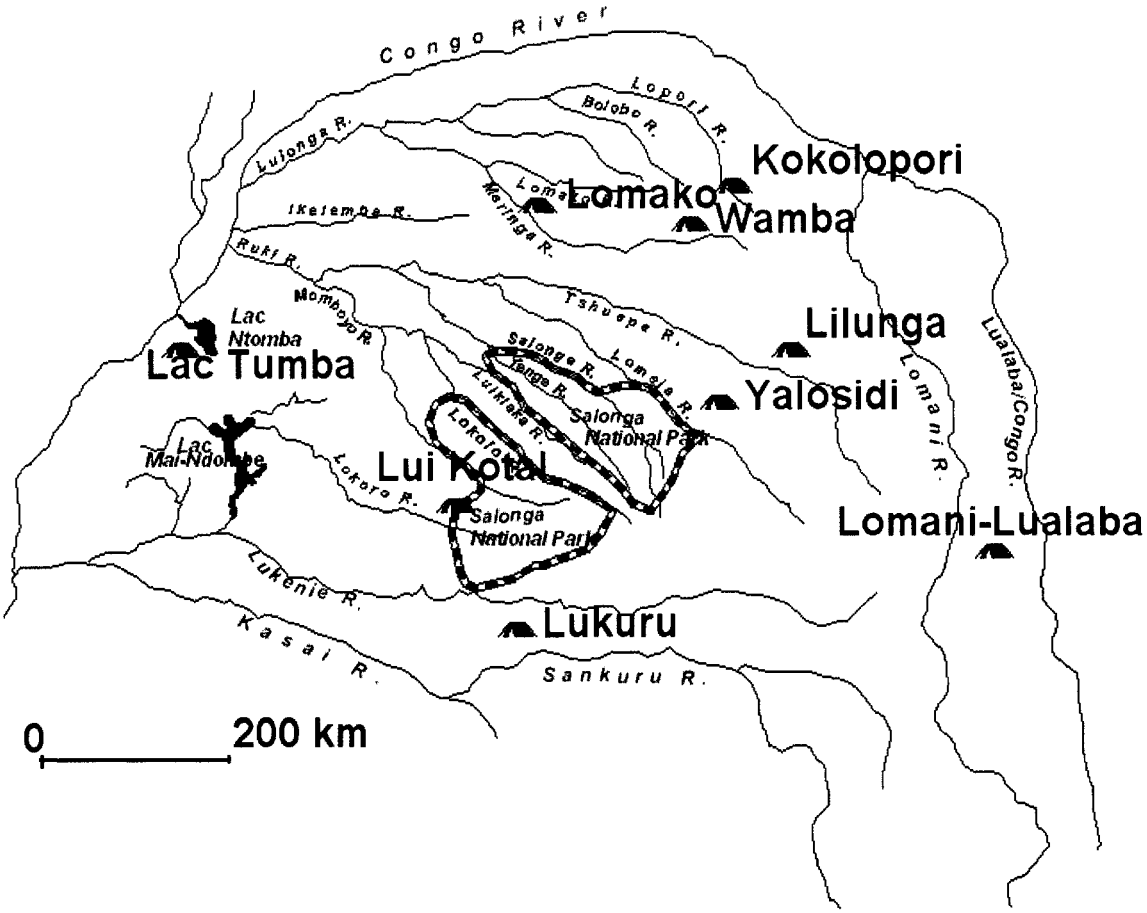

Figure 3. The Distribution of Bonobo Research Sites in DRC. Map based on Thompson et al. (2003). Tent images show approximate location. Site names are marked in blue.

Table 2. Current Proposed Distribution of Bonobo Research Sites Throughout DRC. Information gathered from Thompson et al. (2003).

Site	Affiliated Researchers	Local Threats to Bonobo Conservation	Proposed Activities
Kokolopori	Albert Lokasola	Subsistence hunting Poaching by Military Cultivation Absence of eating taboo	Survey Confirmed bonobos
Lac Tumba (West) Reserve de Mabali	Mbangi Mulavwa Mwanza Ndunda	Subsistence hunting Commercial hunting Logging Agriculture fields Transport access to river	Survey Confirmed bonbos Informal sensitization
Lomako	Jef Dupain	Subsistence hunting Commercial hunting Human immigration	Status assessment International public awareness documentary
Lomami-Lualaba		Commercial Hunting Logging in North Human movement	Reconnaissance survey
Lui Kotal	Jonas Eriksson	Subsistence hunting Commercial hunting Logging Proximate mining	Economic development Research
Lukuru	Jo Thompson	Subsistence hunting Commercial hunting Logging Mining	Economic development Community relations Infrastructure building Reclassify status
Salonga National Park	Inogwabini Bila-Isia Gottfried Hohmann Omari Ilambu Gay Reinartz	Poaching Cultivation Illegal human habitation Proximate logging Proximate mining	Status mapping Ground-truth images Inventory and survey Infrastructure rehab Research
Wamba	Takeshi Furiuchi, Chie Hashimoto, Genichi Idani, Suehisa Kuroda, Yasuko Tahiro,	Subsistence hunting Coffee plantations Agriculture fields	Reestablished research School support Poaching patrols

In 1997, Tutsi and anti-Mobutu rebels, backed by Rwanda, overthrew President Mobutu. Laurent-Desire Kabila was put into place as the new leader, and the country was re-named Democratic Republic of Congo. A year later, Rwanda and Uganda backed forces to remove Kabila from power. They then took over much of eastern sector of DRC (Edgerton 2002), which included a large part of the range of wild bonobos.

Foreign researchers were forced to leave their research stations, and were not able to return for four years. This left the scientific community with many questions about the safety of the bonobos, their co-existing wildlife, and the researchers' Congolese collaborators (Thompson et al. 2003).

During wartime, conflicting factions and refugees did not recognize the boundaries of protected areas. Forested areas were used for shelter, and wildlife was used for sustenance. Along the eastern border of DRC, 410-770 tons of forest products were extracted from the Virunga National Park per day. Park guards were not respected, and their wearing uniform risked them being identified a rebel, which could result in death (Vedder et al. 2001).

Kabila retained control of the southwestern part of DRC, with the assistance of Angola, Zimbabwe, and Namibia. The Lusaka Peace Accord was signed in 1999, in which all countries and rebel groups agreed to a ceasefire. Despite the accord, fighting continued (Edgerton 2002).

In January, 2001, President Laurent Kabila was assassinated. His son, Joseph Kabila, took over the presidency. He immediately began peace talks with all contenders. Foreign researchers were then able to get back into their research sites and do initial assessments of incurred losses. In July 2003, leading bonobo researchers met in Japan in order to pool their most recent information and to formulate strategies for moving forward with conservation efforts. Their population estimates were optimistic (Thompson et al. 2003).

Kabila set up a transitional government in 2003, which allotted political seats to representatives of the rebelling factions and was to lead to a democratic election in July, 2006. However, rebel troops are reported to still occupy much of the eastern region (Consular Information Sheet 2006).

In 2002, a team from the Research Center for Ecology and Forestry (CREF) surveyed the status of the bonobos in the western region (Mwanza et al. 2003). In nine forest blocks, southwest of Lake Tumba, they interviewed people from local ethnic groups and searched for signs of recent bonobo presence. Twenty four out of twenty six people claimed to enjoy eating bonobo meat at least twice a year. Some reported that bonobos were agricultural pests, and some used bonobo parts for traditional medicines. A total of 294 bonobo nests in eight out of nine forest blocks, and a total of 54 bonobos were directly observed in 4 out of 9 forest blocks. The team stated concerns regarding pending logging concessions (Mwanza et al. 2003).

Relative to their ape cousins: the chimpanzees, gorillas, and orangutans, little is known about wild bonobo behavior and evolutionary genetics (Bradley and Vigilant 2002), as is also the case for other mammalian species living in the region (Grubb 2001). This is due to intermittent periods of field access along with the majority of bonobo research coming from three field stations, two of which are only 150 km apart (Thompson 2002). What little is known about bonobo behavioral ecology has yet to be corroborated by studies administered throughout their range. Also, the majority of what has been referred to as bonobo-type behavior has been developed by researchers who

used providing food as a quick method to gain access to the individuals (Thompson 1997).

Threats to the Future of Wild Populations of Bonobos

Habitat Fragmentation

Bonobo habitat is fragmented by both natural barriers, such as rivers, and by human settlements (Kano 1992). Increasing anthropogenic causes of fragmentation could occur at a rate exceeding the rate of natural adaptation to environmental change. Habitat fragmentation creates island-like areas of isolated populations. Isolated populations have limited gene flow between them, which will eventually lead to low gene diversity within isolated populations, and in some cases local extinctions.

Logging Concessions

Habitat fragmentation also occurs when blocks of forest are lost due to logging activity. It was reported (Dupain & Van Elasacker 2001) that in DRC, 86% of the forest cover remains, due to the reluctance of logging companies to invest in DRC because of political instability (Thompson et al. 2003). Logging concessions granted before the civil war were either postponed or no longer recognized. In the state of conflict, many prime logging forests were occupied by rebel factions. It was especially risky for logging companies to make deals with internationally unrecognized institutions. Infrastructure for timber transportation relied predominately on the Congo River, which passes through

territory occupied by the DRC government (Baker et al. 2003). Ironically, the resolution to conflict in DRC may open the nation's pristine forest treasure to exploitation by European and Asian logging companies.

Human Transmitted and Zoonotic Disease

Apes are susceptible to most of the same diseases as humans. Infectious disease transmitted from humans could be devastating to wild populations of apes (Butynski 2001). A landmark case of this occurring in reverse, is the zoonotic origins of the Human Immunodeficiency Virus (HIV). Substantial evidence supports that humans contracted HIV from nonhuman primates in Africa, who are carriers of Simian Immunodeficiency Virus (SIV) (Gao et al. 1999, Weis and Wrangham 1999,Hahn et al. 2000). A recent report (Keele et al. 2006) revealed that SIV was detected in fecal samples from wild chimpanzees in Cameroon. The illegal bushmeat trade (described below) provides the ideal and probable mode of disease transmission; as hunters handle bloody carcasses, and consumers ingest sometimes poorly cooked the meat (Gao et al. 1999, Hahn et al. 2000). Other primates do not appear to develop the complications involved with AIDS; they are simply carriers. Other diseases likely remain undetected thus far and transmission in either direction could mean devastation to either species. The severity of the impact of HIV on the prolific human population illustrates the potential of irrevocable consequences of seemingly benign diseases could have on ape populations.

Commercial Bushmeat Trade and Subsistence Hunting

The illegal commercial bushmeat trade in Africa is the most significant threat to all three African ape populations (gorillas, chimpanzees, and bonobos) (Goodall 1994, Cowlishaw & Dunbar 2000). Adult apes are hunted for their meat. The lower quality pieces are sold to logging camps with thousands of migrant workers, while the higher quality pieces are transported to cities and sold, for elevated prices, to middle/upper class people, who serve it at their tables as a status symbol (Barnett 2000, Ammann 2001).

The commercial bushmeat trade in Africa is an extremely complex problem (Noss 1997, Bowen-Jones and Pendry 1998, Cowlishaw 1999, Robinson et al. 1999, Shogren et al. 1999,Wilkie 2001). The significance of each player varies according to the governing state and local conditions. However, the basic cycle persists, and is propagated by human population increase and desperate poverty, compounded by wealthy companies that are prepared to exploit the conditions in order to gain a profit (Oates 1999).

Bushmeat hunters frequently use wire snares as hunting tools. In a survey of Salonga National Park, after the latest civil war, Reinartz (2003), encountered 71 metallic snares in nine kilometers of transect. Bonobos regularly get caught in snares. Even when they are not killed by the poachers, they often lose or mangle limbs, important for climbing, grooming, gathering food, and maternal care. Such injuries may also cause fatal infections (Noss 1998, Wrangham 2001).

With the recent political instability in DRC, trade routes were cut, so that the long distance commercial trade of bushmeat may have slowed (Reinartz 2003). However, villagers are in a desperate situation in rebel-occupied territories, and are likely feeding

their families on whatever protein source they encounter first in the forest (Reinartz 2003). Many local tribes had taboos against eating bonobo meat, but with massive human migrations and culture change, most of those traditional taboos were forgotten. In some tribes and for some government officials, bonobo meat is considered a delicacy (Thompson 1997, 2001b, Wilkie 2001).

ABC Bonobo Orphanage in Kinshasa

Illegal Live Pet Trade

Young ape orphans lack a profitable quantity of meat, so poachers receive a higher return by taking the orphans to the market to be sold in the illegal pet trade. Few orphans survive the trauma of losing their mothers and the long journeys to the cities, however, some do make it to the market. A small percentage of these orphans are confiscated and turned over to one of the multiple ape sanctuaries throughout Africa (Andre 2001).

ABC Sanctuary

Les Amis des Bonobos au Congo (ABC) bonobo sanctuary, founded and directed by Claudine Andre, is situated just outside the capital city, Kinshasa. The bonobo orphans are in critical condition when they arrive at the sanctuary; however, with medical intervention and immediate bonding with a surrogate human mother, some are able to survive (Andre personal communication).

Andre envisions re-introducing this population of orphans into the wild within the next decade. She intends to re-introduce them as a stable social group and is allowing some of the older females to give birth before their release in order to insure that the females acquire appropriate mothering skills. In March of 2004, one of the females, Etumbe, rescued from a Kinshasa research facility, gave birth to an infant. The infant was named Mbano Ya lola and is reported to be fathered by Makali, who was also rescued from the same research institution (Andre personal communication).

Re-introduction of Apes as an Experimental Practice in Conservation

There is much controversy over the role of sanctuaries for orphaned apes, and even more about the practice of rehabilitation and reintroduction (Teleki 2001). Reintroduction experiments of orangutans, *Pongo pygmaeus,* have taken place for the last 30 years. Initial efforts to reintroduce orangutans exposed essential criteria for responsible reintroduction. The results of the early experiments were optimized to develop a reintroduction experiment that follows closely regulated procedures: minimal human contact; intensive health screening; explicit instruction in foraging techniques; and reintroduction into empty forests (Smits et al. 1995). These basic criteria are recommended for any primate reintroduction.

Following the primary reintroduction principle of the IUCN that the release of captive individuals should contribute to the survival of the species in the wild, a successful reintroduction of 20 individuals to habitat co-existing with wild chimpanzees was reported (Tutin et al. 2001, Goosens et al. 2003). They express caution that their

successful reintroduction was after years on islands, with minimal human contact, and that their circumstances were rare and should therefore not serve as a model for less ideal conditions of reintroduction (Tutin et al. 2001).

Relation to State of Knowledge of the Field: Applications of genetic information to conservation management

Understanding the genetic make-up and diversity of a species is a useful tool for developing conservation strategies. Genetic analyses can identify populations that are isolated from other populations, which results in reduced gene flow and can lead to inbreeding fixation of genotypes, and/or low genetic diversity. Genetic diversity is frequently measured by calculating a population's heterozygocity (Freemon and Herron 2001). The heterozygocity is the probability that an individual in a population will be heterozygotic at a given allele rather than homozygotic. Populations with high heterozygocity are considered to be more viable, more likely to survive stochastic events than populations with low heterozygocity. This is because populations that have more variation are more likely to have an allele or a combination of alleles that help the individuals that have those alleles survive through selective pressures such as environment change or disease outbreaks (Cowlishaw and Dunbar 2000).

Conservation biologists use heterozygocity as a tool for making decisions about which populations or species need the most protection. Methods have also been developed to deduce the amount of gene flow between various populations that are potentially isolated. Small, isolated populations usually result in inbreeding, which leads to a lowered heterozygocity, because the reduced number of genotypes increases the

likelihood that like genotypes mate with each other. This results in fewer genetic variants to withstand the pressures of natural selection (Freemon and Herron 2001).

Published Studies of Genetic Research Performed on Wild Born Bonobos

Wild populations of bonobos are situated in a discontinuous distribution, and studies of the isolated populations should reveal interesting differences in morphology and behavioral ecology (Kano 1992). Little research has been published about the genetic make-up of bonobos throughout their distribution (Bradley and Vigilant 2002).

Microsatellites

Microsatellites are short tandem repeats of 2-6 nucleotides found in nuclear DNA. They usually exhibit a lot of variation within a species, because they have a high rate of mutation (10^{-3} events per locus, per generation). They are useful to use when looking at the genetic structure of populations within a species, but are limited in use between species (Hancock 1999).

Microsatellite studies applied to the conservation of wild bonobos are very limited but do provide information that can be useful for further studies. A study examining the heterozygocity of 28 microsatellites in 14 wild born bonobos, from western zoos, found three distinct clusters, which did not coincide with the reported geographic segregation (Reinartz 1997, Reinartz et al. 2000). Using different microsatellite loci, another study was able to establish the likely paternity of individuals from the Eyengo community, in the Lomako forest (Gerloff et al. 1999) (Figure 3). A recent study confirmed the high

genetic diversity of bonobos, from the central region, in a report on 12 microsatellites on the Y chromosome. It also demonstrated the expected low male-regulated gene flow, resulting from female migration (Eriksson et al. 2006).

Mitochondrial DNA

Mitochondrial DNA (mtDNA) is maternally inherited. In chimpanzees and bonobos, mitochondrial DNA is theoretically useful for defining populations' outer boundaries (Goldberg 1996, 1997), given the fact that females migrate, while males remain philopatric to their communities.

Mitochondrial DNA was compared between two study sites, located about 150 kilometers apart (Eyengo community of the Lomako Forest and E1 group at Wamba study site), in order to support behavioral observations that females migrate (Hashimoto et al. 1996, Gerloff et al. 1999).

A comparison of the mitochondrial sequences of bonobos from the eastern, central, and southern parts of their range (Figure 4, Table 3) to the published sequences (Gerloff et al. 1999, Hashimoto et al. 1996) from the northern parts of their range was the first study to begin the inventory of genetic information for bonobos (Eriksson et al. 2004). The aim of their study was to search for evidence of populations isolated by the numerous river barriers that intersect bonobos' range. The results revealed no evidence for significant barriers to gene flow between the southern, central, and northern populations, however, the eastern population (note: a sample of only 15 individuals) did show lower nucleotide and haplotypic diversities. Phylogenetic analysis, using neighbor-

joining methods, divided the haplotypes into two clusters (Eriksson et al. 2004). Thirty-five haplotypes from the northern, central, and southern distributions were distributed within both clusters, but all of the five eastern haplotypes grouped closely into only one of the clusters. Their study suggested that the genetic make-up of the central population was representative of the mitochondrial diversity of bonobos in general. Therefore this central population should be considered as a potential conservation priority (Eriksson et al. 2004). Salonga National Park already exists within the range of the central population (Figure 4).

Project Background

This study looks at the variability in the HV1 region of the mitochondria in the ABC bonobos. Though this region in the genome is noncoding and is not suspected to be submitted to the pressures of natural selection, it is used to model expected trends in the remainder of the genome and for understanding the genetic make-up of the wild bonobo population.

The exact origins of the bonobos from the Amis des Bonobos du Congo (ABC) sanctuary are unknown, as they are victims of the bushmeat trade. However, the general regional origins of each orphan have been reported, and this information is useful in developing models for the expected genetic make-up of the wild population. The ABC orphans are genetic representatives of their source populations, and provide insights for conservation strategies. The reported geographic origins of the ABC bonobos indicate that 12 bonobo orphans are from the north/central region; 16 bonobo orphans are from

the western region; and 3 bonobo orphans are from the southern region (Figure 4). The origins of the remaining eleven individuals are unknown.

Though the presence of bonobos in the western part of their proposed range was confirmed (Mwanza et al. 2003), studies have not yet been published on the genetic make-up, behavioral repertoire, or ecology of the western population. As the ABC orphan population consists of 16 individuals with reported western origins, a mtDNA analysis of the viability of the western population is done in this study, using the 16 orphans as genetic representatives of their source population. These results are compared to published studies from the northern population (Gerloff et al. 1999 and Hashimoto et al. 1996), and with the eastern, southern, and central populations (Eriksson et al. 2004).

This study additionally provides an analysis of the structure of the upcoming artificially selected population to be re-introduced to the wild in the near future. This man-made population will likely become the source of a new wild population and will potentially interbreed with already-established wild populations, introducing a unique compilation of genes. The potential genetic consequences of this act are considered below.

Project Objectives

This study has three main objectives:

•To provide the first initial assessment of the genetic structure of wild bonobos living in the western limits of distribution.

•To explore various hypotheses for the evolutionary history of bonobos, especially considering the distinct lineages that appear in published results (Eriksson et al. 2004, Hashimoto et al. 1996, Gerloff et al. 1999).

•To evaluate the genetic prospects of the ABC population as a founding population in the wild, through the process of re-introduction.

Project Hypotheses

The following hypotheses were developed:

- Distinct mitochondrial DNA haplotypes exist in each of the five regions examined.
- The source populations of ABC bonobos with unknown origin can be identified by matching their mitochondrial haplotypes to the mitochondrial haplotypes of ABC bonobos with known geographic origins.
- Extensive homogeneity is expected in the mitochondrial DNA of the ABC orphan population.

Table 3. Definitions of Bonobo Ranges Used in this Study. River lengths are from Beadle (1981).

Northern Clade •Isolated by Maringa/Lulonga River to south (732 km long) •Isolated by Lualaba/Congo River to north
Central Clade •Isolated by Maringa/Lulonga River to north •Discontinuous distribution to south
Western Clade •Isolated by Lukenie River in south (1069 km long)•Isolated by discontinuous distribution in north
Southern Clade •Isolated by the Lukenie River in north •(1069 km long) •Limited by Kasai/Sankuru Rivers in south
Eastern Clade •Isolated by Lomani River to West (1448 km long) •Isolated by Lualaba/Congo River to East

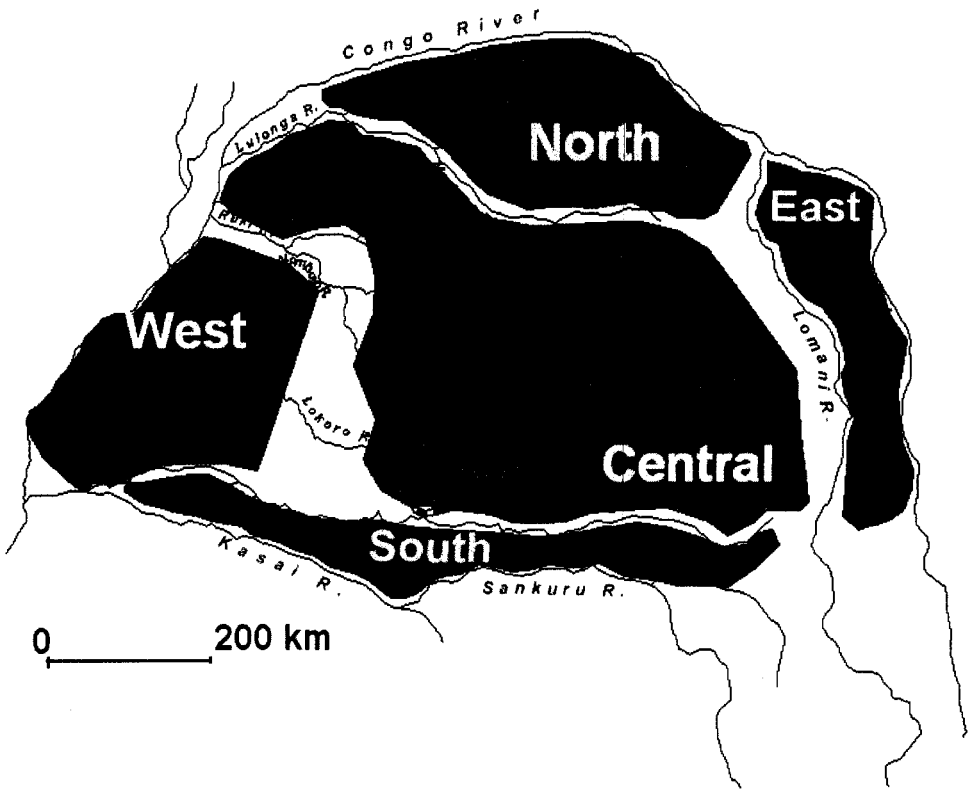

Figure 4. Map of Division of Distribution of Bonobo Ranges Used in this Study.

MATERIALS AND METHODS

Study Subjects

The mtDNA of 42 wild born orphaned bonobos were sequenced for this study (Table 4). The sample consists of 22 males and 20 females, aged between two and nineteen years old. The majority of the orphans were captured in the wild between 1995 and 2004. The exact geographic origins of these individuals are not known, as most were confiscated at the local markets. However, records of estimated origins do exist for 29 individuals (Table 4).

The haplotypes of the orphans were compared to five published haplotypes of 42 wild bonobos from Lomako (Gerloff et al. 1999); seven published haplotypes of 17 individuals from Wamba (Hashimoto et al. 1996); and 28 published haplotypes of 104 individuals from the eastern (N=15), southern (N=26), and central (N=63) parts of bonobo range (Eriksson et al. 2004). Sixteen of the ABC orphans had reported origins from locations in the western parts of the bonobo range. In this study they were considered as genetic representatives of their western source populations. In all, the analysis examined the mtDNA of 205 bonobos.

Table 4. Samples From This Study. Name; Sex; Estimated Age in 2004; Cities of Reported Geographic Origins; Region of Origin (North=N, Central=C, South=S, West=W, xx=unknown); Approximate Month and Year of Date of Capture in Wild; and Date of Arrival at ABC Sanctuary. INRBxx and GPPxx =Research Centers and Unknown Origins.

	Name	Sex	Est. Age 2004	Reported Geographic Origins	Region Of Origin	Date of Capture in wild	Arrival date at sanctuary
1	Mikeno	M	14	Basankusu	C	10/94	9/12/94
2	Bukavu	M	11	Lomela	C	11/95	4/20/96
3	Manono	M	11	Mushie	W	5/97	9/5/97
4	Boende	M	8	Kutu	W	1/96	4/20/96
5	Tatango	M	10	Basankusu	C	4/97	7/11/97
6	Inongo	M	7	Inongo	W	1/98	3/17/98
7	Kikwit	M	5	Boende	C	2/00	4/21/00
8	Bondo	M	4	Oshwe	S	4/00	1/9/00
9	Beni	M	5	Bolobo	W	3/99	3/17/00
10	Matadi	M	4	Kutu	W	4/01	5/2/01
11	Mimia	F	19	?	x	6/82	4/6/01
12	Maya	F	12	Bikoro	W	9/92	8/15/95
13	Opala	F	10	Boende	C	11/97	12/22/97
14	Semendua	F	9	Oshwe	S	8/98	11/11/99
15	Bandundu	F	6	Lukolela	W	1/99	11/11/99
16	Salonga	F	6	Bolobo	W	9/98	3/17/00
17	Kalina	F	5	Befori	C	9/99	10/26/99
18	Isiro	F	5	Lokolama	C	11/00	1/25/00
19	Kisantu	F	5	Kutu-Nioki	W	1/01	4/6/01
20	Lukaya	F	4	Basankusu	C	3/02	4/5/02
21	Nioki	F	4	Nioki	W	4/00	5/15/00
22	Luputa	F	2	Nioki	W	8/21	10/16/01
23	Fizi	F	5	Mushie	W	11/02	3/15/03
24	Tembo	M	7	Bulukutu	C	3/00	10/9/03
25	Dilolo	M	3	Nioki	W	4/06	5/15/03
26	Lisala	F	3	xx	xx	xx	4/10/04
27	Likasi	F	3	xx	xx	xx	4/10/04
28	Bili	F	3	Bokungu	C	6/03	10/7/03
29	Etumbe	F	xx	INRBxx	xx	xx	2/29/04
30	Ruzizi	F	2	Nioki	W	1/04	2/28/04
31	Lulua	F	3	xx	xx	xx	3/19/04
32	Tshilomba	F	xx	INRBxx	xx	xx	2/29/04
33	Max	M	18	GPPxx	xx	xx	4/10/04
34	Virunga	M	2	Inongo	W	2/04	5/14/04
35	Tex	M	7	GPPxx	xx	xx	4/10/04
36	Mixa	M	4	GPPxx	xx	xx	4/10/04
37	Api	M	4	Boende	C	5/03	10/27/03
38	Lipopo	M	4	Panu (Idiofa)	S	8/03	10/28/03
39	Makali	M	xx	INRBxx	xx	xx	2/29/04
40	Kiri	M	2	Bikoro	W	3/04	4/5/04
41	Keza	M	xx	INRBxx	xx	xx	2/29/04
42	Kikongo	M	4	Boende	C	1/04	4/20/04

Sample Collection

Fecal samples were collected non-invasively by Crispin Mahamba, ABC sanctuary's biologist, using standard precautions to avoid contamination. Approximately 3-5 grams of feces were scraped from the surface of fresh fecal samples, using gloves and a sterile applicator, and then placed into sterile, labeled polypropylene tubes containing desiccating silica beads. Two to four samples were collected from each individual, on different days. The samples were shipped to San Jose and stored in the original containers, in desiccant beads, in a dark closet at room temperature.

DNA Extraction

DNA extraction was performed using a Qiagen QIAamp DNA Stool Mini Kit, following the manufacturer's instructions. In order to avoid contamination (especially because human primers were used) and in order to avoid zoonotic transmission of potential pathogens, all extractions were performed in an isolated room, dedicated uniquely to this procedure (Taberlet et al. 1996, 1999). In addition, safety goggles, masks, gloves, and lab coats were worn during the handling of the feces. Negative controls were performed with each extraction and then run through subsequent PCRs to check for contamination. Contamination was not detected at any stage of testing.

PCR Amplification

In order to compare the results of this study with other studies, primers L15997 (5'-CACCATTAGCACCCAAAGCT-3') and H16498

(5'CCTGAAGTAGGAACCAGATG-3') were used (Gerloff et al. 1999). Primers were prepared in an area dedicated uniquely to primer preparation (Taberlet et al. 1996, 1999). All PCR preparations were carried out under a hood, which was sterilized with UV light between preparations. DNA was loaded in a separate, distinct area.

Twenty-five µl PCR reactions were prepared with 4.0 µl DNA, 0.50 µM each of forward and reverse primers, 1X PCR buffer (which had an additional 1.5 mM Mg^2), 3 µl $Mgcl^2$ (25 mM Mg^{2+}), 0.1 U BSA, 0.20 mM each dNTPs, and 1.0 U Taq DNA polymerase (Mastertaq Kit, Eppendorf). As recommended (manufacturer's protocol Qiagen Mini Stool Kit) BSA was added. Negative controls were prepared for each PCR reaction, with DNA grade water replacing the DNA, to verify the purity of the reagents and procedures. PCR reactions were performed in a Mastercycler Personal Thermocycler (Eppendorf), for 1 minute each at 94°c, 60°c, and 72°c, for 30 cycles (Gerloff et al.1999).

Agarose Gel Electrophoresis

The success of PCR amplifications was verified by running 5 µl of PCR product on 2% agarose gels, alongside a 100 bp ladder (Biolabs). The purity of PCR products was also checked by running the negative controls. After staining the gels for 30 minutes with ethidium bromide, the results were visualized on a Gel Doc 1000 Image Analyzer (BioRad).

Sequencing

Before sequencing, PCR products were purified using QIAquick PCR Purification Kit (Qiagen). Samples were sequenced using primer L15997. Samples prepared in the summer of 2003 were sequenced at San Jose State University on Applied Biosystems 310. Samples, received in 2004, were sequenced at Sequetech, a private biotechnology company in Mountain View, California, using an Applied Biosystems 3730 DNA Analyzer. All sequences were prepared using a D rhodamine dye kit (Applied Biosystems).

Data Analyses

In order to address the three main objectives of this study, data was analyzed in two different stages. First, the sequences of the ABC orphans were analyzed as a single population so that questions about re-introduction of the group as the source of a future wild population could be examined. Second, the sequences of the ABC orphans with reported western origins (ABC West) were considered as representatives of the western part of the wild bonobo range and thus compared to the published sequences (Hashimoto et al. 1996, and Gerloff et al. 1999, Eriksson et al. 2004,) representing bonobos from the southern, central, northern, and eastern parts of their range.

The frequencies of the haplotypes from the Eriksson et al. (2004) study were not reported (representing eastern, central, and southern regions). Therefore, as outlined in Table 5, analyses that require frequency values were performed on the samples from the whole ABC population, ABC West, and the North (Hashimoto et al. 1996, and Gerloff et

al. 1999). When possible, the results of these analyses were then compared to the results of the analyses reported by Eriksson et al. (2004). It should be noted that Eriksson et al. (2004) treated the samples from the northern region as two separate populations: North, from the Lomako study site (Gerloff et al. 1999); and Northeast, from the Wamba study site (Hashimoto et al. 1996). As Gerloff et al. (1999) reported no evidence that these two populations were genetically isolated, this study treated them as one population. This does lead to a gap in distance values between the North/Northeast and the East, Central, and South, when compared to the distance values between the North and West. This was handled by providing additional cells in tables reporting results and by labeling Eriksson et al. (2004)'s North as "NorEr" and Northeast as "NeaEr."

Table 5. Outline of the Analyses Performed on Various Populations and Groupings. "✓" indicates that analysis was done for given population. "Er" indicates that values were taken from Eriksson et al. (2004). Dark cells indicate that analysis could not be performed on given data set or would not be informative to objectives of this study. Abbreviations for analyses are defined in the text below. Populations are abbreviated as follows: ABC-all ABC bonobos; ABC West –bonobos with reported western origins; All Hap –haplotypes from all regions; No –north; Cen –Central; S –south; E –east; N –north (Gerloff et al. 1999); NE – northeast (Hashimoto et al. 1996). Two groups listed together in a column indicate inter-group comparisons.

	ABC	ABC West	All Hap	No	Cen	S	E	West &No	Cen &S	Cen &E	S &E	N & others	NE	All Erik
N	**42**	**16**	**41**	**53**	**63**	**26**	**15**	**69**	**89**	**78**	**41**	**36**	**17**	**157**
H freq	✓	✓		✓				✓						
Diversity Measures														
π	✓	✓		✓	Er	Er	Er					Er	Er	Er
K	✓	✓		✓	Er	Er	Er					Er	Er	Er
h	✓	✓		✓	Er	Er	Er					Er	Er	
D Mat	✓													
Population Divergence Indices														
F_{ST}								✓	Er	Er	Er	Er	Er	
Da								✓	Er	Er	Er	Er	Er	
AMO								✓						Er
Nm								✓						
Demographic Measures														
Taj. D								✓						Er
Mis dist								✓						Er
t								✓						?
Phylogenetic Methods														
MJN			✓					✓						
NJT	✓		✓											

Various software, all available on the internet, were used at each stage of analysis. Sequences were aligned by hand, using BioEdit version 7.0.5.2 (Hall 1999). Phylogenetic Analyses were performed using MEGA version 3.0 (Kumar et al. 2004). DnaSP version 4.10 (Rosas et al. 2003) and Arlequin version 2.0 (Schneider et al. 2000) were used for haplotype determination and statistical analyses.

All maps in this study were created using Map Maker Gratis (Dudley and Map Maker 2006). Butynski's (2001) map of bonobo distribution and *National Geographic Atlas of the World* were used as references for plotting rivers and cities. ABC haplotypes

were presented on a map of bonobo distribution (Figure 5), according to their reported origins.

Genetic Variation

Heterozygocity of the HV1 region was determined by calculating haplotype (h) and nucleotide (π) diversities using Arlequin 2.0 (Schneider et al. 2000) and DNAsp 4.0 (Rozas et al. 2003). Haplotype diversity is an estimation of the probability that two haplotypes selected randomly from a population will be different (Nei 1987). Nucleotide diversity is calculated as the mean number of pairwise nucleotide differences at each locus (Tajima 1993).

Genetic Distance

Genetic distance between the reported western population and the northern populations was inferred using various methods and then compared to values reported by Eriksson et al. 2004). Pairwise F_{ST} values were calculated using Tamura-Nei (1993) to determine the average distance within and between populations. F_{ST} values range between 0 and 1. An F_{ST} score of 1 would indicate that gene exchange is not occurring between populations. F_{ST} scores closer to 0 indicate that there is little difference in the genetic make-up between the populations and that therefore there is likely recent and frequent migration between the populations. Nei's (1987) Da method is a measure of the net number of nucleotide differences between regions. Estimates of female-mediated gene flow (Nm) were calculated using Lynch and Crease (1990) methods, where NST, the same as F_{ST} is obtained using Jukes and Cantor (1969)correction. An Nm value of 1 would indicate that

one female migrates between populations per generation, which is sufficient to avoid genetic differentiation by genetic drift (Slatkin 1987). An Nm estimate less than 0.5 may connote limited gene flow between populations.

AMOVA (Analysis of Molecular Variance) ("Amo" in Table 5) analysis was performed using Arlequin 3.01 (Excoffier et. al 2005). This calculation determines the percentages of variance both within and between populations. Covariance components of intra-individual differences, inter-individual differences, and inter-population differences are determined using hierarchical analysis of variance. These covariance components are used to compute fixation indices, whose statistical significance is then tested by non-parametric permutation (Excoffier et al. 1992).

Tests of Neutrality and Demographic History

Tajima's D (D) was performed on all ABC sequences and all North sequences in order to reveal the likelihood that the evolution of the HV1 region in bonobos has evidence of recent population expansion. Using the infinite site model, the number of segregating sites and the mean pairwise differences should be close to equivalent. Divergence from this expectation could be an indicator of differences in mutation rates among sites; selection; or unstable populations (Tajima 1989).

Fu and Li's D and F statistics were calculated in order to compare the number of remote mutations to the number of recent ones (Fu and Li 1993). The difference between Fu and Li's D and F tests is that the D test considers the differences between the number of singletons and number of mutations, and the F test considers the differences between

the number of singletons and K, the mean pairwise distance between haplotypes. With both tests, a positive value implies that there have been few recent mutations, therefore the potential that balancing selection has been occurring.

Using DNAsp v.4.0 (Rozas et al. 2003), pairwise number of differences in nucleotide sites were calculated and displayed in a graph of mismatch distribution. If the distribution displays multiple peaks, it indicates that the population has likely stayed at a relatively constant size. However, if the graph shows a unimodal distribution, it may indicate a recent population expansion, which could signify a prior bottleneck (Slatkin and Hudson 1991).

Performance of the mismatch distribution provides the expansion parameter τ, which was employed, according to Roger and Harpending (1992), to calculate the date of the population expansion. The time of the recent bottleneck (t) was estimated by $\tau=2ut$: $u=\mu k$; μ is the mutation rate per site per year; and k is the sequence length. The mutation rate of 7.5% per million years (Tamura and Nei 1993) was used.

Minimum Spanning Network

A minimum spanning network was created using median joining methods in Network 4.111 (Bandelt et al. 1999). This procedure displays the mutational steps and probable missing links (median vectors) between haplotypes. It is useful for visualizing the relationships between haplotypes. Transversion to transition ratio was set at 1:1, and all sites were weighted equally.

Phylogenetic analyses

Phylogenetic trees were created as graphic images of the hypothetical evolutionary relationships. Trees were rooted using mitochondrial sequence of *Pan troglodytes verus*, GenBank accession AF176721 (Deinard 1997).

RESULTS

ABC Bonobos

Four hundred and fifteen base pair HV1 sequences were identified from 42 individuals. Polymorphism was detected at 47 sites. Nucleotide diversity measured π = 0.0229; SD = 0.00318 (Nei 1987). A single base pair "c" insertion was observed in five individuals (haplotype abcE). This insertion appears in a highly variable strand, creating a strand of seven c's, with the consensus being six c's.

The sequences divided into 14 haplotypes (Table 6), based on single base pair differences. Haplotypes are labeled using "abc" to designate haplotypes from the ABC orphanage, and the letters A-N to distinguish between haplotypes determined by single base pair differences (Table 6). When present, the number in parentheses indicates the number of individuals assigned the given haplotype. In order to portray the geographic distribution of haplotypes, ABC haplotypes were plotted, in red, near the reported cities of origin on a map of their region (Figure 5).

Table 6. Assignment of Haplotypes to ABC Bonobos. Haplotypes are capital letters A-N. Bonobos are assigned region codes according to their reported geographic origins. INRBXX and GPPXX indicate individuals from a local research center (with unknown wild origins). "XX" means unknown.

[illegible]	[illegible]	[illegible]	[illegible]
A	Semendua	Oshwe	South
	Keza	INRBXX	XX
B	Kalina	Befori	Central
	Bondo	Oshwe	South
C	Api	Boende	Central
D	Manono	Mushie	West
	Salonga	Bolobo	West
E	Tatango	Basankusu	Central
	Lisala	XX	XX
	Max	GPPXX	XX
	Inongo	Inongo	West
	Boende	Kutu	West
F	Virunga	Inongo	West
	Makali	INRBXX	XX
G	Mixa	GPPXX	XX
	Matadi	Kutu	West
	Kikwit	Boende	Central
	Mikeno	Basankusu	Central
	Bandundu	Lukolela	West
H	Bili	Bokungu	Central
I	Luputa	Nioki	West
J	[illegible]	[illegible]	Central
	[illegible]	Bikoro	West
	[illegible]	XX	XX
	[illegible]	Bolobo	West
	[illegible]	[illegible]	Central
	Ruzizi	Nioki	West
	[illegible]	XX	XX
	Kikongo	Boende	Central
	Fizi	Mushie	West
	[illegible]	XX	XX
	Kiri	Bikoro	West
K	Lukaya	Basankusu	Central
	Nioki	Nioki	West
L	Kisantu	Nioki	West
M	Opala	Boende	Central
N	Dilolo	Nioki	West
	Tex	GPPXX	XX
	Etumbe	INRBXX	XX
	Tshilomba	INRBXX	XX
	Bukavu	Lomela	Central
	Lipopo	Panu (Idiofa)	South

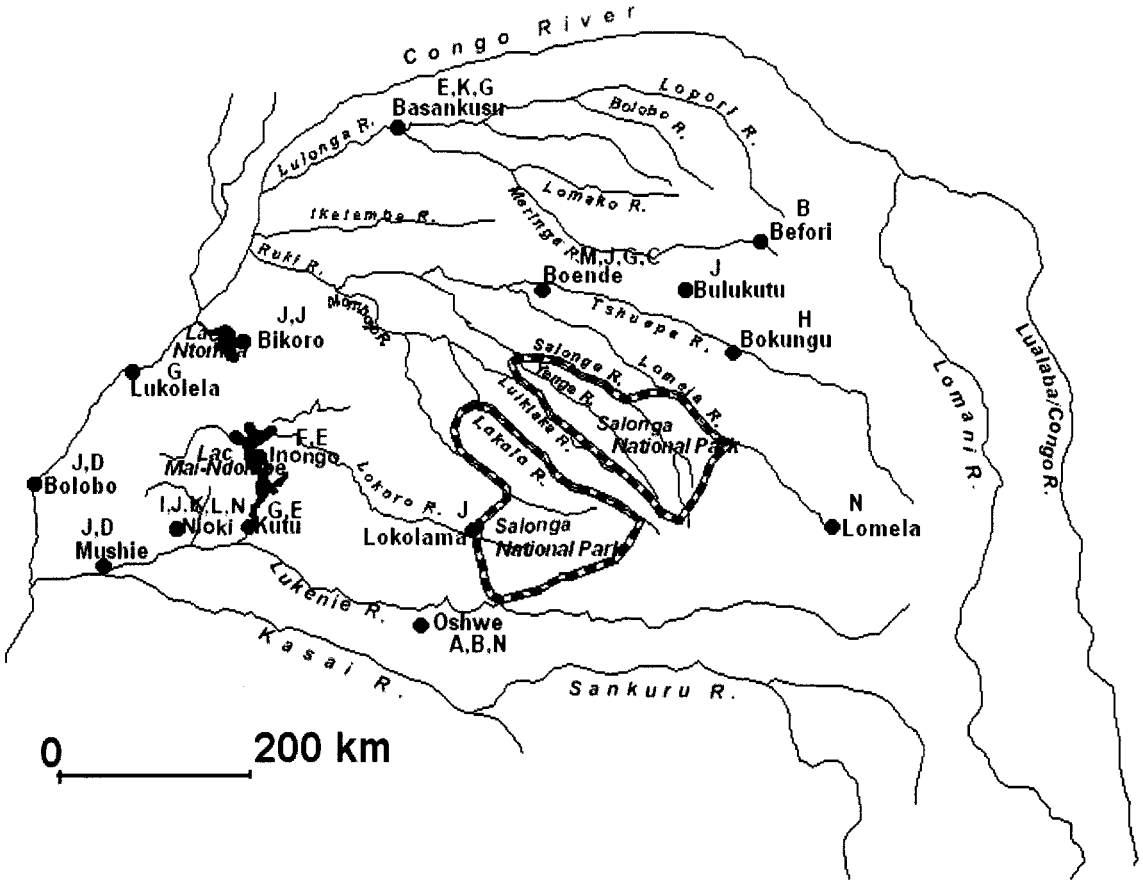

Figure 5. Map of Bonobo Range with ABC Haplotypes Assignments. Haplotypes are plotted in regions of the orphan bonobos' reported origins.

The haplotype diversity of ABC's bonobos was calculated as h = 0.890; SD 0.029 (Nei 1987) (Figure 6). Haplotype abcJ was the most frequently observed haplotype; assigned to 11 bonobos, and making up 27% of the observed haplotypes. Fourteen percent of the bonobos showed haplotype abcN and both abcE and abcG appeared 12% of the time.

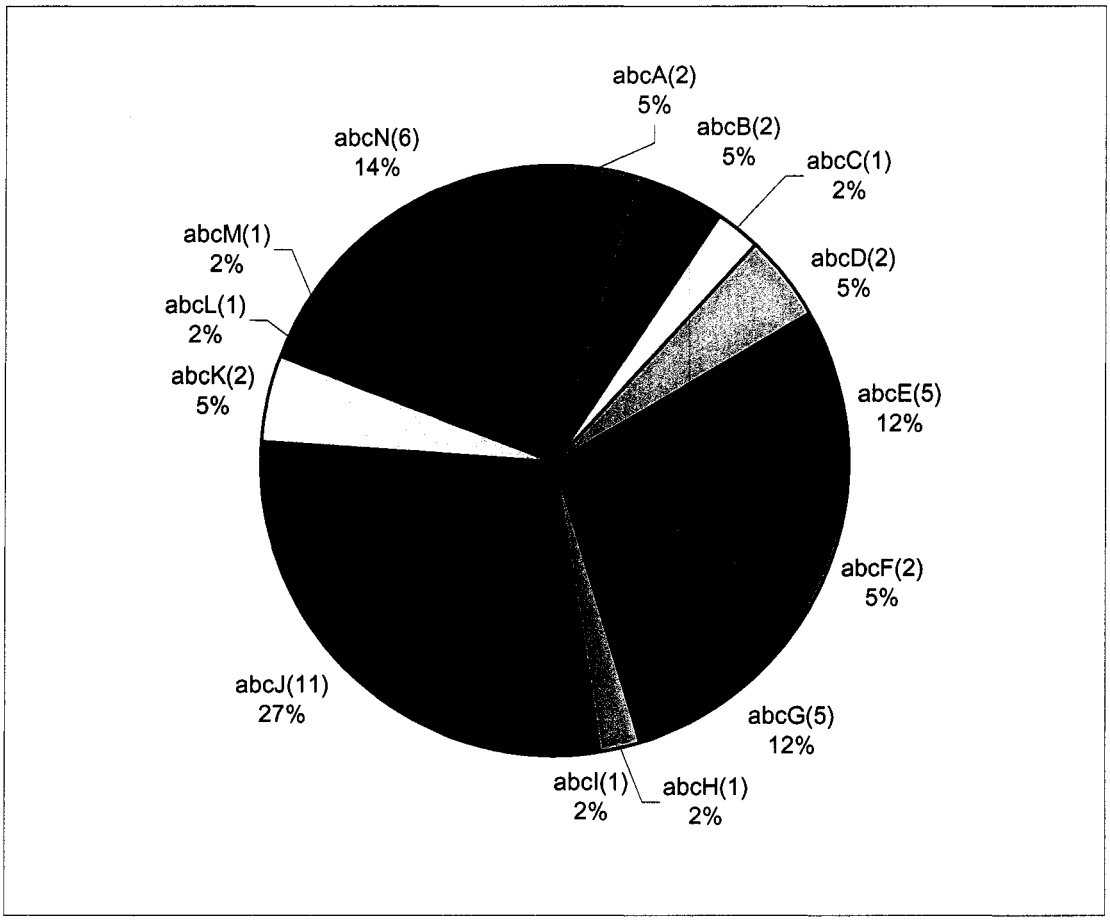

Figure 6. The Haplotype Frequencies of ABC Bonobos.

The pairwise distances between ABC haplotypes are shown (Table 7). AbcA-C, are more closely related to each other than they are to haplotypes abcD-N. AbcD, assigned to two individuals, shows distances ranging from 0.040, as with abcK and 0.058, as with abcC. This haplotype forms its own cluster in phylogenetic analyses (Figure 7). Both individuals with abcD have reported origins in the south-western portion of the western distribution (Figure 5).

Table 7. Pairwise Distances Between ABC Haplotypes. Distances were calculated using Kimura 2-parameter in Mega 3. Letters along diagonal line indicate abc haplotype designations.

abcA(2)	**A**											
abcB(2)	0.002	**B**										
abcC(1)	0.010	0.012	**C**									
abcD(2)	0.048	0.050	0.058	**D**								
abcE(5)	0.048	0.051	0.059	0.045	**E**							
abcF(2)	0.048	0.051	0.059	0.045	0.020	**F**						
abcG(5)	0.053	0.056	0.064	0.051	0.010	0.015	**G**					
abcH(1)	0.051	0.053	0.061	0.053	0.038	0.022	0.032	**H**				
abcI(1)	0.051	0.053	0.061	0.048	0.017	0.007	0.012	0.025	**I**			
abcJ(11)	0.045	0.048	0.056	0.043	0.017	0.002	0.012	0.025	0.005	**J**		
abcK(2)	0.043	0.045	0.053	0.040	0.020	0.005	0.015	0.022	0.007	0.002	**K**	
abcL(1)	0.053	0.056	0.064	0.051	0.025	0.010	0.020	0.032	0.012	0.007	0.010	**L**
abcM(1)	0.053	0.056	0.064	0.051	0.025	0.010	0.020	0.032	0.012	0.007	0.010	0.005 **M**
abcN(6)	0.053	0.056	0.064	0.045	0.025	0.015	0.025	0.038	0.017	0.012	0.015	0.015 0.015

Phylogenetic analysis (Figure 7), using neighbor joining method with Tamura-Nei parameters, with 1000 bootstrap replicates, segregated ABC haplotypes into 3 clusters; labeled 1, 2, and 3. *Pan troglodytes verus* is used as an outgroup.

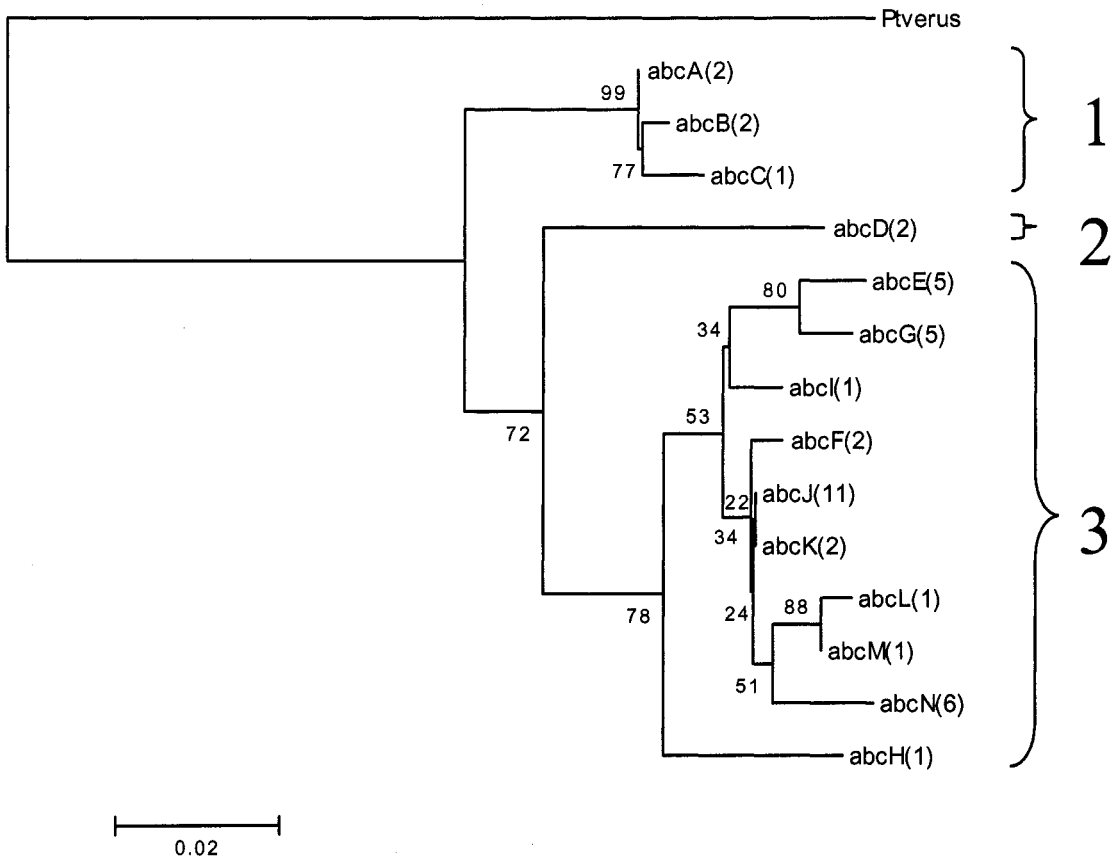

Figure 7. Neighbor Joining Tree of ABC Haplotypes. Created using Mega 3 (Kumar et al. 2004), using Tamura-Nei model (Tamura and Nei 1993). *Pan troglodytes verus* is used as the outgroup. Numbers at nodes represent boostrap values after 1000 replications. Numbers in parenthesis represent number of individuals with shared haplotype. Numbers 1-3, adjacent to brackets, represent the 3 clusters recognized.

ABC West

Though plotting the haplotypes of the individuals with reported geographic origins (Figure 5) does not show strict geographic segregation of the haplotypes, a specific trend is worth further exploration. In the western region, there is a high concentration of abcJ, making up 5 out of 16, or 31% of the observed haplotypes. Apart from 2 occurrences of abcD, which forms a clade of its own, the remaining local haplotypes group in the same cluster in phylogenetic analyses (Figure 7) and show relatively small pairwise distances, ranging from 0.002-0.017 (Table 7).

This observation led to an analysis of the levels diversity of ABC West population (Table 8). With 415 usable base pairs, nucleotide diversity (π) is 0.018675, mean pairwise difference (K) is 7.75, and haplotype diversity (h) is 0.8917.

In subsequent sections of this study, the number of base pairs used in analyses is reduced to 285, in order for the results of this study to be comparable to the other concerned published sequences. In this case, the western population's diversity levels shifted. Nucleotide diversity (π) is 0.026053, mean pairwise difference (K) is 7.425, and haplotype diversity (h) was 0.8500. This reduction in sequence also condensed haplotypes abcJ and abcK into a single haplotype: abcJK.

Table 8. List of Bonobos, Haplotypes, and Cities of Origin of ABC West. The column of consolidated haplotypes is in reference to upcoming section of results and represents incidents of identical haplotypes with published studies.

#	Bonobo	ABC Haplotype	Consolidated Haplotype	Reported Origins
1	Manono	abc D	abcD	Mushie
2	Salonga			Bolobo
3	Inongo	abc E	abcE	Inongo
4	Boende			Kutu
5	Virunga	abc F	C10abcF	Inongo
6	Matadi	abc G	C2abcG	Kutu
7	Bandundu			Lukolela
8	Luputa	abc I	abcI	Nioki
9	Maya	abc J	C4abcJK	Bikoro
10	Beni			Bolobo
11	Ruzizi			Nioki
12	Fizi			Mushie
13	Kiri			Bikoro
14	Nioki	abc K	C4abcJK	Nioki
15	Kisantu	abc L	abcL	Kutu-Nioki
16	Dilolo	abc N	C5abcN	Nioki

Consistent with Figure 6, haplotype abcJK makes up a large proportion (37 %) of the haplotype occurrences, representing six individuals. The remaining haplotypes, each of one or two individuals, are relatively evenly spread through the spectrum (Figure 8).

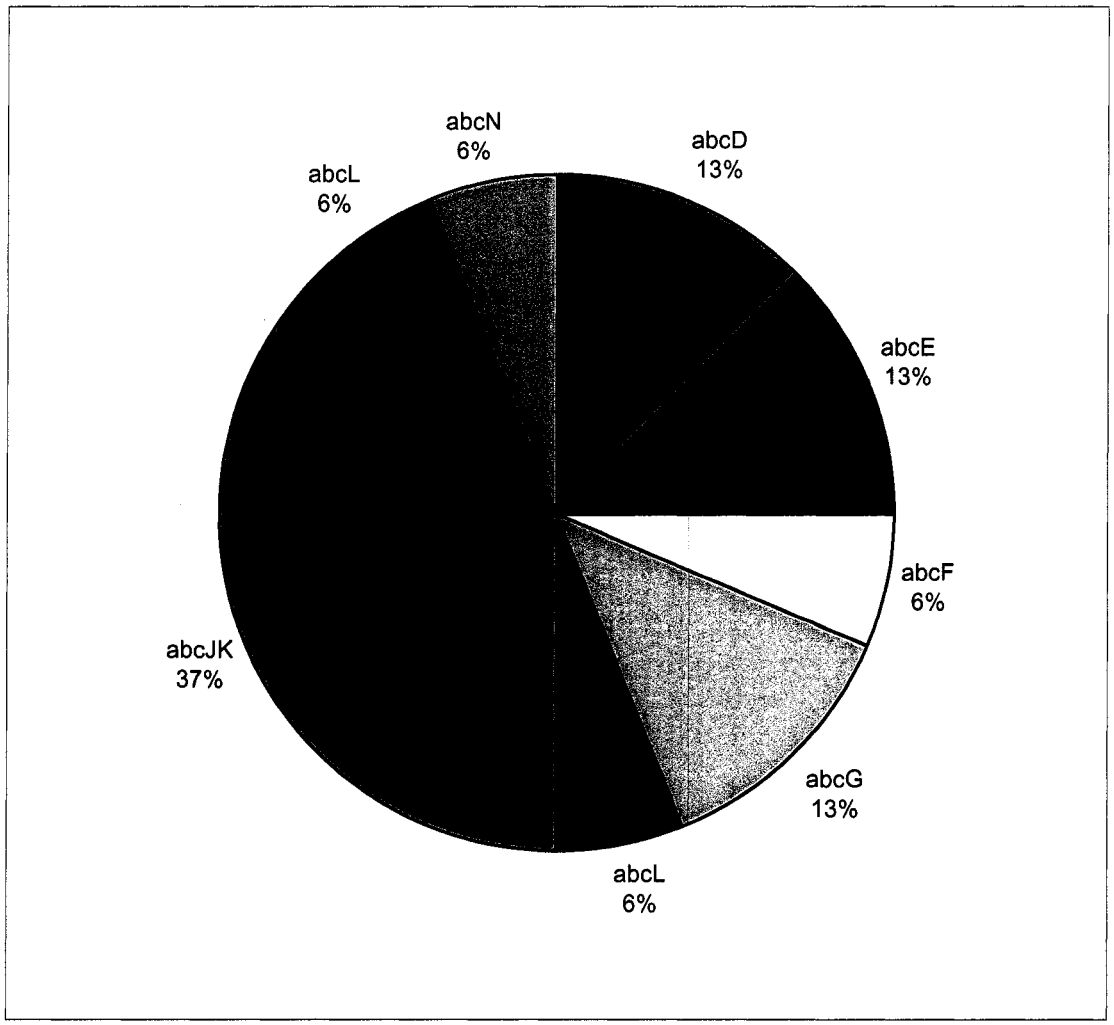

Figure 8. The Frequency of Haplotypes of ABC West.

Phylogenetic analysis (Figure 9) demonstrates that two clusters, from the overall ABC population (Figure 7), are represented in the western population, but not the third (cluster 1). Nucleotide distance between the two clusters, using Tamura Nei model in Mega 3, was calculated to be 0.063.

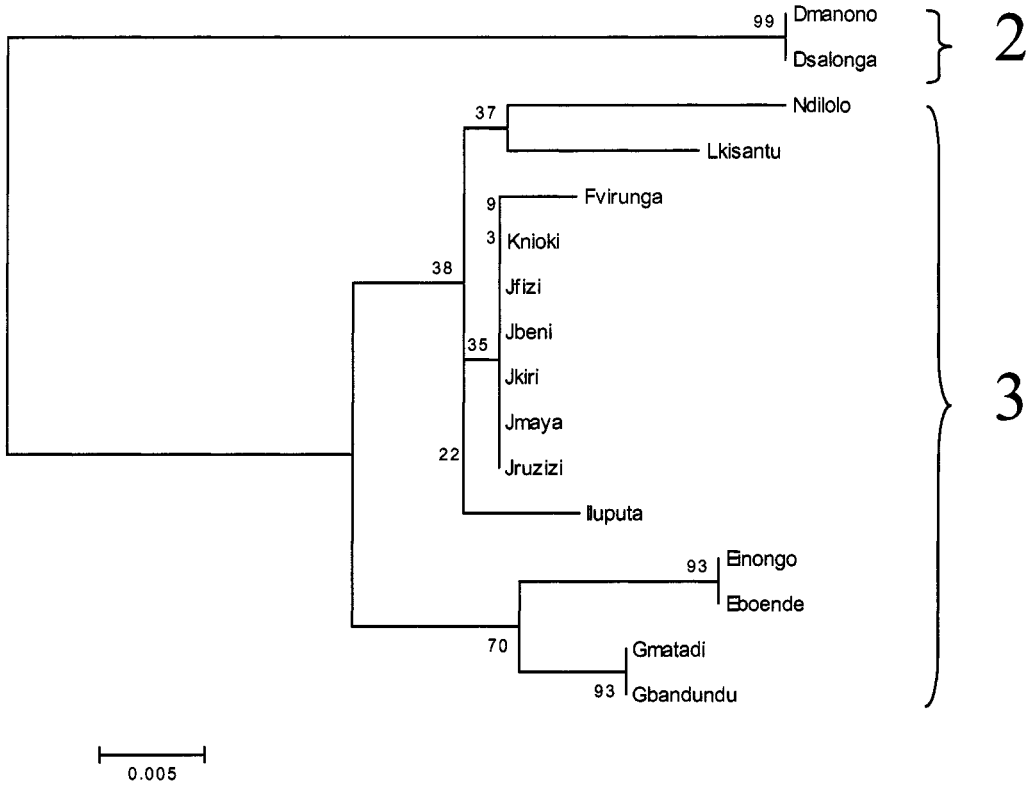

Figure 9. Neighbor Joining Tree of ABC West. Tree was created using Tamura Nei Methods. Bootstrap values are indicated at nodes. Each bonobo from the western region is defined by a capital letter that indicates the assigned haplotype and then their name. Clusters are labeled with brackets, using same identification as Figure 7.

ABC Bonobos Compared to Published Sequences of Wild Bonobos

When sequences from previous studies (Hashimoto et al.1996, Gerloff et al. 1999, Eriksson et al. 2004) were combined with the sequences from ABC orphans, the data set was reduced to 285 base pairs. The most variable areas of sequence were conserved in the analysis. Seventy two polymorphic sites were observed.

Four out of eight ABC West haplotypes were identical to published haplotypes from the central region, and did not match haplotypes from the north, east, or south. One haplotype from the north was identical to a haplotype from the central region and one was identical to the south. All eastern haplotypes were unique. Seven out of fourteen ABC haplotypes were identical to published haplotypes.

Consolidation of the 56 haplotypes into identical haplotypes, from the original 199 individuals, resulted in 41 haplotypes (Table 9). Matching haplotypes were named with original haplotype names listed sequentially.

Comparisons between regions of nucleotide diversity (π), average pairwise difference (K), and haplotype diversity (h) are reported (Table 10). Diversity values for overall ABC population are similar to those reported for other regions, apart from the East. However, ABC West has lower diversity values, which resemble those reported for the East.

Table 9. Consolidation of Matching Haplotypes of All Studies Involved in This Project. Numbers in parentheses are known numbers of occurrences for each given haplotype.

ABC Haplotype	North Wamba (Hashimoto et al. 1996)	North Lomako (Gerloff et al. 1999)	**Central (C), South (S), & East (E) (Eriksson et al. 2004)**	**Consolidated Name For This Study**
			C1	C1
abcG(5)			C2(11)	C2abcG
			C3	C3
abcJK(13)			C4	C4abcJK
abcN(6)			C5	C5abcN
			C6	C6
			C7	C7
			C8	C8
			C9	C9
abcF(2)			C10	C10abcF
			C11	C11
			C12	C12
			C13	C13
			C14	C14
			C17	C17
	KM (5)	EY3(6)	C18	C18EY3KM
			E1	E1
			E2	E2
			E3	E3
			E4	E4
			E5	E5
			S1	S1
			S2	S2
			S3	S3
			S4	S4
			S5	S5
abcA(2)	NO(3)	EY2(7)	S6	S6abcAEY2NO
abcB(2)			S7	S7abcB
abcC(1)	MT(1)			abcCMT
abcD(2)				abcD
abcE(5)				abcE
abcH(1)				abcH
abcI(1)				abcI
abcL(1)				abcL
abcM(1)				abcM
	BH(1)	EY1(9)		EY1BH
		EY4(7)		EY4
		EY5(7)		EY5
	HI(1)			HI
	IK(2)			IK
	SN(4)			SN

Table 10. Diversity Values for Populations of Bonobos. Values with "*" are values determined in Eriksson et al. (2004). "N" = sample size; π = nucleotide diversity (Nei 1987); K= average number of nucleotide differences (Tajima 1993); h= haplotype diversity (Nei 1987).

Population	N	π	K	h	# haplotypes
Overall	199				41
ABC	42	0.030	8.540	0.8688	13
ABC West	16	0.025	7.192	0.85000	8
North	53	0.040	11.621	0.8614	9
Central	63*	0.032*	10.3*	0.923*	16*
South	26*	0.030*	9.5*	0.813*	26*
East	15*	0.023*	7.8*	0.781*	15*
NorEr (Wamba)	36*	0.038*	12.1*	0.819*	5*
NeaEr (Lomako)	17*	0.031*	9.8*	0.781*	7*

Phylogenetic analyses, using neighbor-joining methods, divided the haplotypes into three clusters, representing three maternal lineages (Figure 10). The clusters were labeled 1, 2, and 3, following the same groupings as in Figure 7.

Haplotypes representing the central and southern regions were distributed throughout the tree, showing similar occurrences in both clusters. Northern haplotypes remain exclusive to cluster one, apart from EYBH1 in cluster three and HI in cluster two. Eastern and Western haplotypes are both exclusive to cluster three.

A median joining network (Figure 11), constructed using all haplotypes, showed trends comparable to the phylogenetic tree using the same data set (Figure 10). Three clusters were created; each separated by approximately 14 mutations from cluster 3. According to this constellation, cluster 1 and cluster 2 each branch off of cluster 3 and are separated from each other by approximately 26 mutations. In cluster 1, haplotype S6abcA has 7 branches, linking all other haplotypes in cluster 1. It is interesting to note

that all individuals assigned to S6abcA have southern origins (see discussion). In cluster 2, haplotype C4abcJK serves as a link to all other haplotypes in cluster 3; including the more distantly placed haplotypes for the eastern region. It also provides branches, through median vectors, to clusters 1 and 2.

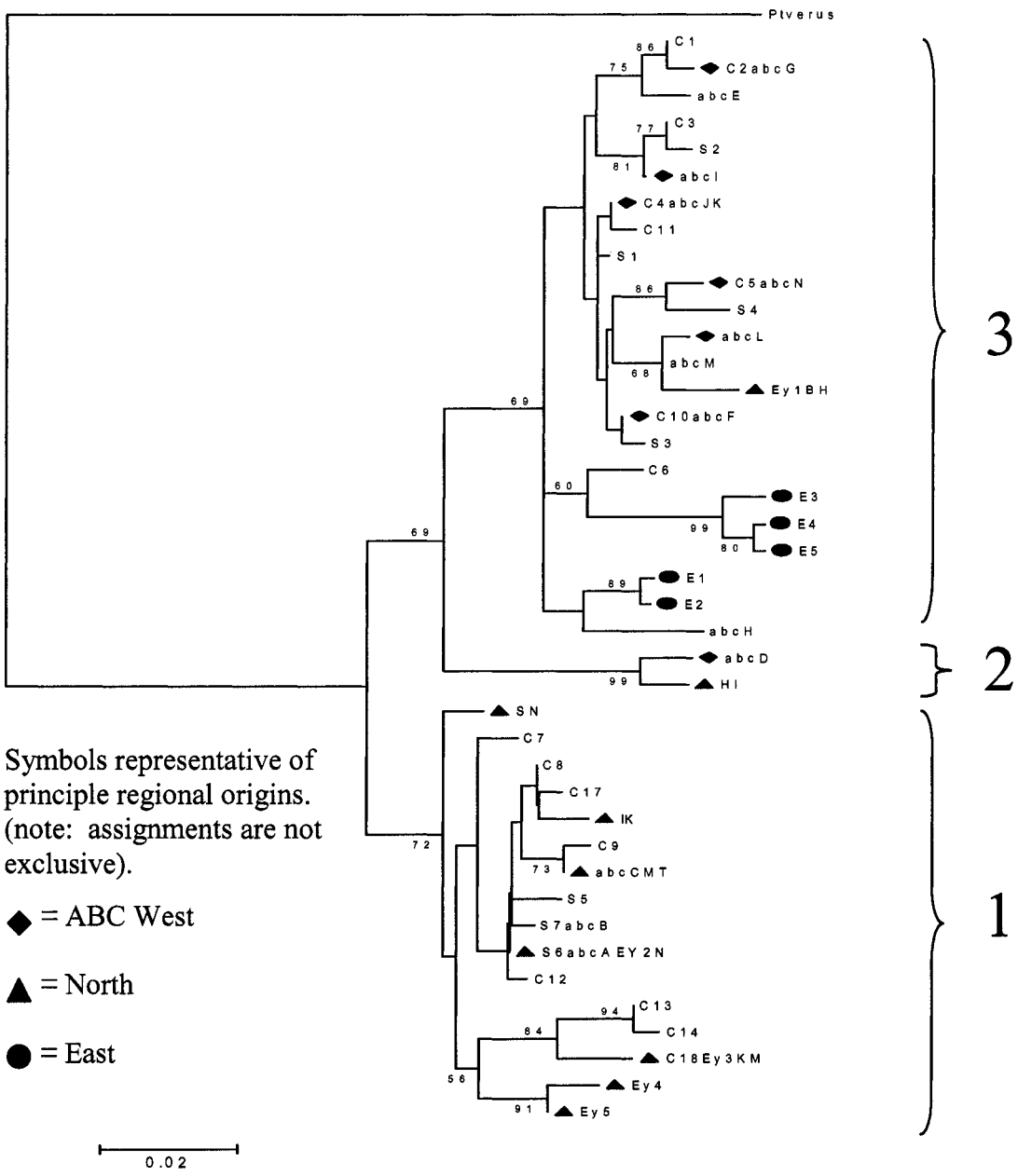

Figure 10. Neighbor Joining Tree of All Haplotypes. Tree was created in Mega 3 (Kumar et al. 2004), using Tamura-Nei model (Tamura and Nei 1993). *Pan troglodytes verus* is used as an outgroup. Numbers at nodes represent bootstrap values after 1000 replications. Numbers 1-3, adjacent to brackets, demonstrate the partition of 3 clusters, and are the same groupings as in Figure 7.

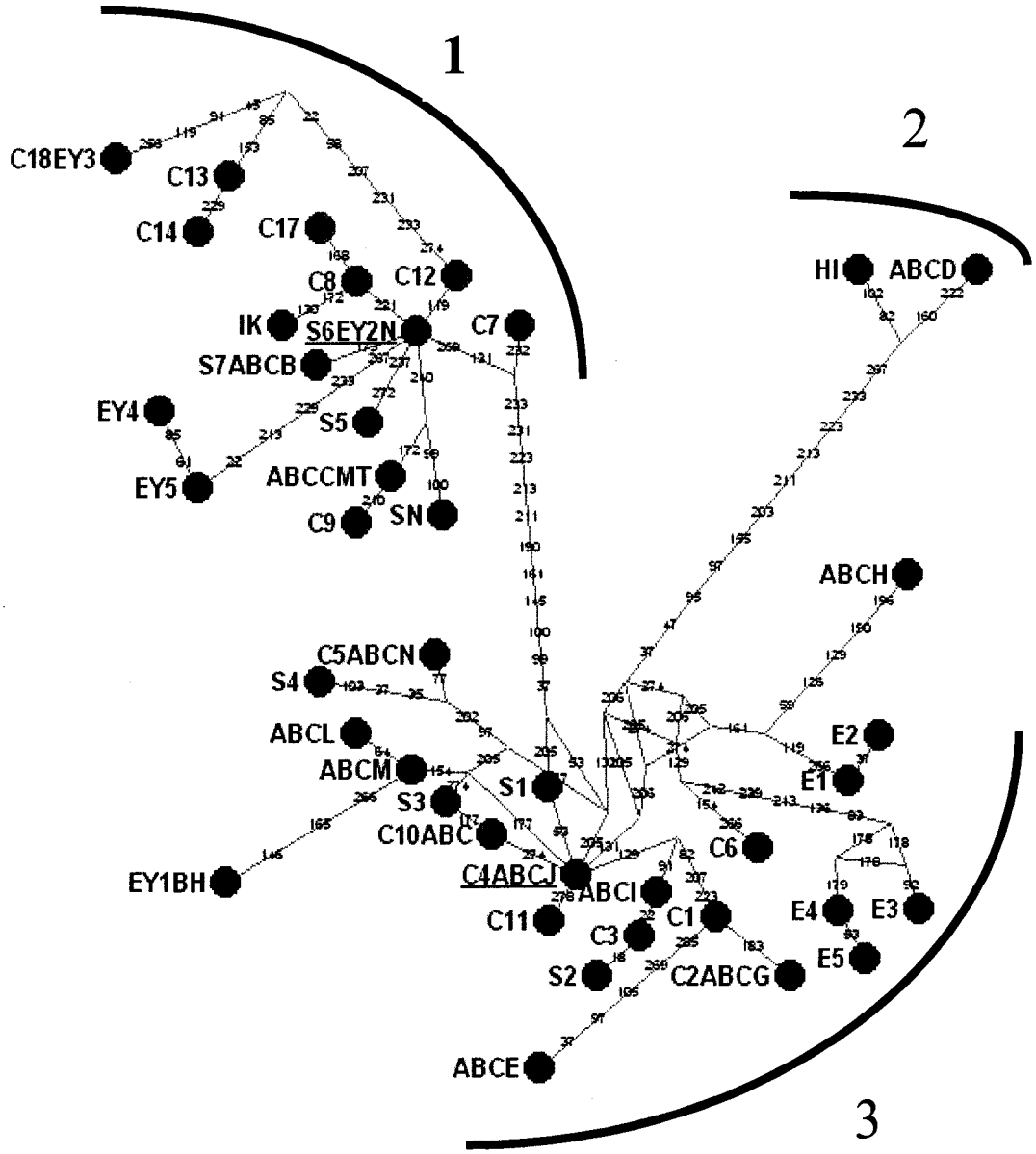

Figure 11. Median Joining Network of all haplotypes. Numbers along links represent mutations. Lengths of links are not proportional to distance. ● = ABC West; ● = Central ; ● = South; ● = North; ● = East; Bi-colored nodes indicate haplotypes shared between regions. Clusters are indicated 1, 2, and 3.

Distances Between ABC West and the Northern Population

As haplotypic frequencies were available from the published sequences of the northern range (Hashimoto et al 1996, Gerloff et al.1999), further analyses were possible. These analyses are especially interesting because they compare two populations that appear to be both geographically and genetically isolated (separated by a series of river systems). Additionally, phylogenetic analyses also split these two populations into separate clades. This section explores statistical significance of the potential geographical structuring of these two populations, based on the HV1 gene. It compares the F_{ST} and Da values to those previously reported (Eriksson et al. 2004) (Table 1) .

Pairwise F_{ST} distance values between various populations (Table 11, Figure 12) relatively corresponded with the values provided by Eriksson et al. (2004). F_{ST} distance between ABC West and the North population was calculated to be 0.4302 and between ABC and the North to be 0.3505. Eriksson et al (2004) reported the greatest overall F_{ST} values to be between the east and other regions (0.489 with north; 0.638 with northeast; 0.35 with central; and 0.419 with the south), which is to be expected because of the significant geographic migrating distance between these locations (around the Lomani River). The smallest F_{ST} values reported by Eriksson et al. (2004) were between the northern populations and central and southern populations, where there appears to be a recent and direct migration route.

Nei's (1987) Da were figured for all ABC as compared to the North as 0.01916 and ABC West and North as 0.02501 (Table 11).

Table 11. Distance Values of Between Populations. Below the diagonal, pairwise F_{ST} values using Tamura-Nei distance calculations. Values with "*" are values determined in Eriksson et al. (2004). Above the diagonal line are Da values (Nei 1987).

Population	ABC	ABC West	North	NorEr	NeaEr	Central	South	East
ABC	X	X	0.01916					
ABC West	X	X	0.02501					
North	0.3505	0.4302	X					
NorEr				X	1.64*	3.86*	2.13*	9.98*
NeaEr				0.117*	X	6.73*	4.13*	12.8*
Central				0.252*	0.427*	X	0.92*	4.5*
South				0.138*	0.316*	0.083*	X	6.0*
East				0.489*	0.638*	0.307*	0.419*	X

Nst values were calculated as 0.4334 for the North and ABC West; and 0.3534 for the North and all ABC bonobos. Nm (number of migrating females per generation) was reported as 0.65 between ABC West and North; and 0.91 between all ABC and North.

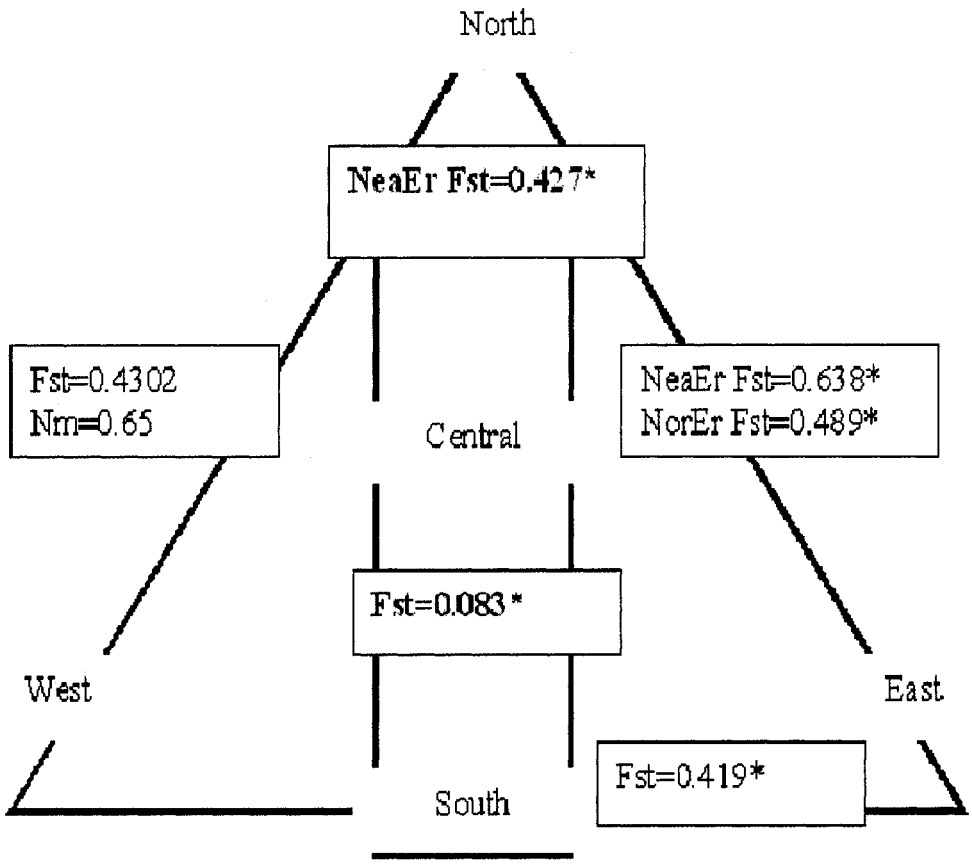

Figure 12. Diagram of F_{ST} Values Between Populations. "*" indicates values reported by Eriksson et al. (2004). Given the lack of frequency values, distance values for the west are limited to west and north comparisons. Nm values are also reported between the north and west.

AMOVA analysis (performed in Arlequin 3.01) revealed that 39% of variation appeared among populations, while 61% is distributed within the populations (Table 12).

Table 12. Results of AMOVA Analysis of Genetic Structuring Between North and ABC West.

Source of Variation	Degrees of Freedom	Sum of Squares	Variance Components	Percentage of Variation
Among Populations	1	86.898	3.38	38.51
Within Populations	63	339.932	5.4	61.49
Total	64	426.831	8.7	
Fixation index F_{ST}: 0.38506				

The median joining network for ABC West and the North (Figure 13) shows similar clustering as the median joining network for the whole set of haplotypes (Figure 11). It shows the number of individuals with each haplotype and emphasizes the central locations of haplotypes EY2 and ABCJK.

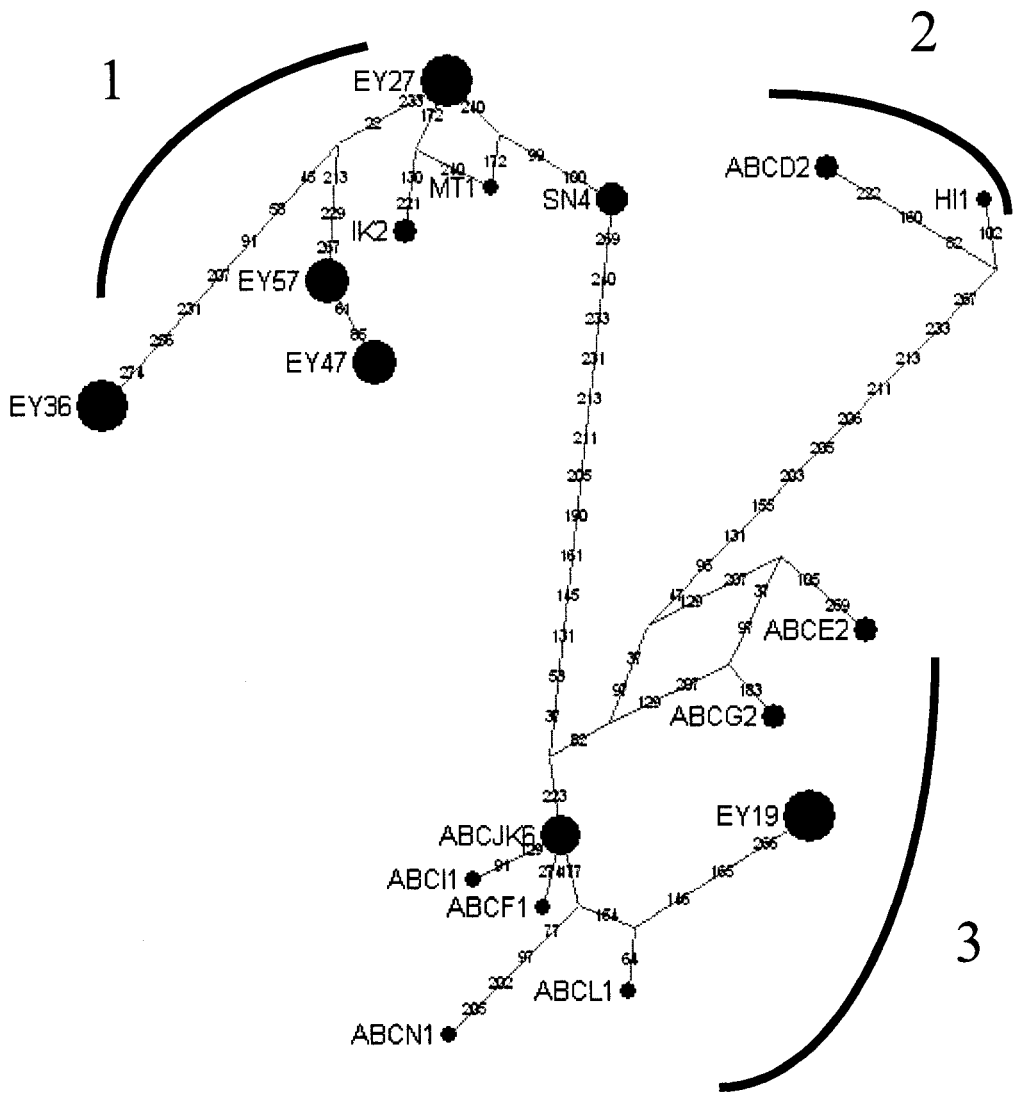

Figure 13. Median Joining Network with ABC West and North. Numbers along links represent mutations. Lengths of links are not proportional to distance. ● = ABC West; ● = North. The size of the circles is proportional to number of individuals sharing haplotype, and the final number is the number of individuals with given haplotype. Clusters are indicated 1, 2, and 3.

Historical Demography of ABC Bonobos and North

In order to further investigate the evidence that the HV1 region provides about historical bonobo population stability and migration, all ABC sequences and all northern sequences were combined to represent the wild bonobo population. As frequencies were not available from Eriksson et al. (2004), they are not included in this survey.

Tajima's D analysis, on all ABC sequences and all sequences from the north, provided a value of 0.7427 and was not significant ($p>0.10$). This result indicates no evidence for sudden population expansion and is corroborated by the results from mismatch distribution performed on the same data set (Figure14). The multimodal distribution of the graph suggests no evidence of recent population bottlenecks or expansions.

The Tau value ($t= 7.082$) was figured through mismatch distribution and was employed to estimate the time to the most recent bottleneck; $t = 165,660$ years. This corresponds with an epoch of 21 glacial and non-glacial cycles; resulting in cycles of forest expansion and retreat (Table 1) (Hamilton and Taylor 1991).

Both Fu and Li's F (1.1713) and D (1.1848) showed no significance ($p > 0.10$) (Table 13). These results imply no evidence for a high number of recent mutations, suggesting that the population is currently in equilibrium.

Table 13. Summary of Demographic Parameters Calculated with All ABC + North.

π	K	t (years)	Tajima's D	Fu's & Li's F	Fu's & Li's D
0.04567	12.97156	165, 660	0.74270 not significant P>0.10	1.1713 not significant P>0.10	1.1848 not significant P>0.10

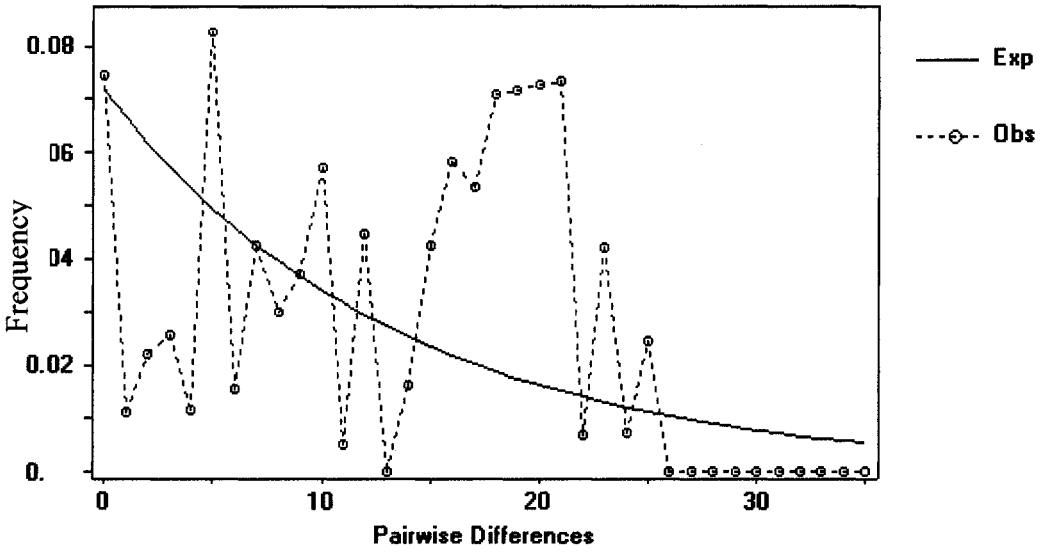

Figure 14. Mismatch Distribution of all ABC Sequences Combined with Sequences from Northern Population. Tau est. 7.082. Solid line curve represents expected curve is population is in expansion or decline.

DISCUSSION

This study extends our understanding of bonobo population genetics. An analysis of the HV1 region of the previously unstudied western region of the wild bonobo range is provided. Using genetic traces, combined with studies from various disciplines, speculation is made into both recent and historical migration routes. These results, although not conclusive, are applied to discussion regarding current issues affecting the reintroduction of the ABC bonobos, and the future of wild bonobo populations.

Implications for Reintroduction of ABC Bonobos into the Wild

A responsible decision to re-introduce any population of animals into the wild must be based on a highly complex set of parameters. The contribution that genetic studies can make to this process is invaluable. Re-introduction creates the founding of a new wild population, which could eventually interbreed with already existing wild populations. Understanding the genetic make-up of the founding population can help managers to avoid or compensate for the negative effects of inbreeding and outbreeding depression.

A founding population with too little variability lacks the diversity of alleles that increases the population's chance of surviving diseases and stochastic events in their environment (Freeman and Herron 2001). Additionally, interbreeding between like genomes increases the likelihood that recessive alleles with deleterious effects are combined. This increases the proportion of individuals with non-adaptive traits, eventually leading to inbreeding depression.

If a re-introduced population interbreeds with an already intact wild population, this could introduce genes that are not adaptive to the given environment, this is known as outbreeding depression. For this reason a careful assessment of genetic distances between populations should be performed prior to re-introduction into the range of wild populations.

Viability of ABC Bonobos as Founding Population

The variability found in the HV1 region of the ABC population was similar to the variability reported in the most variable intact wild populations (north, central, and south) (Eriksson et al. 2004). Nucleotide diversity (π) in the ABC population is 0.030; north is 0.040; central is 0.032; south is 0.030. Haplotype diversity (h) of the ABC population is 0.86. Haplotype diversity in the north is 0.86; the central is 0.92; and the south is 0.81. If samples of the various regions are representative of their actual population, then a founding population with similar genetic variability and population size, in good habitat is expected to have a similar chance of viability.

Phylogenetic analyses distributed ABC haplotypes into three clusters, which also resembled the distribution of haplotypes (Figure 7) within the central and southern regions (Eriksson et al. 2004). These results imply that the HV1 genetic composition of the ABC population would provide a healthy foundation for a founding population. Ideally, further studies on other areas in the genome should be done to corroborate these results, as the HV1 region is only a small representation of the overall make-up of the genome.

Potential Concerns About ABC Bonobos Interbreeding With Wild Populations

Because of the political instability in the DRC, the choice of locations for the reintroduction of ABC bonobos has not been obvious. Ideally, all the political, geographical, and ecological concerns would contribute to a strategic policy regarding the best location for ABC bonobo reintroduction (Smits et al. 1995, Tutin et al. 2001). To date, various options have been considered including locations ranging across and outside of wild bonobo distribution (Andre personal communication).

This study evaluates the positive and negative aspects of locales for bonobo re-introduction into the general cardinal regions outlined in this thesis. It considers both genetic and geographic implications, and its analysis is based on the available information. A caveat is in order as this study is limited by analysis of one small locus of the genome; a relatively small sample size; the lack of solid geopolitical policies.

Evidence from the HV1 region does not indicate a high risk for either inbreeding or outbreeding depression arising from the interbreeding of ABC bonobos with any of the wild populations. ABC orphans and the central population show five identical haplotypes, which comprises 26 (62%) of the ABC population. ABC haplotypes were distributed throughout the phylogenetic tree, in a similar pattern as central and southern haplotypes. Distances between the populations, measured in F_{ST}, provided similar scores between all compared populations (apart from the exceptionally low distance between the central and south). AMOVA between the north and western populations showed that 61% of the variation exists within the populations and 38.51% exists between the

populations. This indicates that some degree of population structuring has occurred between the North and West, but that the populations are not distinct. Eriksson et al. (2004) reported similar findings with AMOVA analysis between the northern, southern, eastern, and central populations resulting in 71.68% of variation occurring within populations and 30.61% occurring among regions.

According to the information revealed by the HV1 region, the ABC population comprises individuals that most resemble the central population. Therefore, if it is anticipated that the ABC bonobos will eventually come in contact with wild populations, based on this narrow survey, the ideal locale for reintroduction is within the range of the central region. However there is little evidence to indicate concern over their introduction to the other regions.

Although this study has provided evidence, there is not a high anticipated risk of the introduction of distant haplotypes into the already existing wild populations; risks involving other parameters could be high. Therefore, much consideration should be given before ABC bonobos are introduced to wild habitat and especially in regards to risks inherent in immediate contact with wild bonobos.

Previous primate re-introduction studies have provided a long list of necessary parameters for successful re-introduction (Lardeux-Gilloux 1995, Smits et al 1995). The question of highest priority is to what degree of certitude can we assure that the ABC bonobos do not harbor an undetected disease that could be transmitted to wild populations?

Lardeux-Gilloux (1995) emphasizes the importance of appropriate socialization in order for orangutans to be successfully re-introduced to the wild. All apes are highly social animals and rely heavily on their mothers and other adults for instruction on survival skills in the wild (Goodall 1996, Boesch and Boesch 2000). The ABC bonobo orphans lost their mothers at very young ages. Are these bonobos prepared with the required resilience and the knowledge base required for self-sustenance in unfamiliar terrain? Will they have methods for dealing with the dangers they will encounter: poisonous snakes; predators; and poachers? Will they stick together as a social unit? Do they have the social skills to handle encounters with other bonobo communities?

Future Role of ABC Bonobo Sanctuary

Wildlife sanctuaries serve a variety of purposes that contribute directly to both humanitarian treatment and the conservation of wildlife. They rehabilitate and provide welfare and protection to bonobos that would have died in dire circumstances. They provide employment and education to local residents and international publicity to the plight of the wild bonobo population (Andre personal communication). Wildlife sanctuaries also provide a reservoir of genetic and medical information concerning wild populations. This type of information about cognitive and behavioral repertoires and life histories create a knowledge base for effective conservation planning.

A study on the western population of wild bonobos would have been problematic without the existence of ABC Bonobo Sanctuary. At the time of the sample collections (2002-04), it was quite difficult to obtain access to the field. Therefore, obtaining

samples, representing the western population of bonobos, would have been unfeasible. At present, this endeavor continues to be prohibitive, due to political uncertainty and the lack of understanding of the distribution of the wild bonobos in the western range. This study offers a clue to understanding the genetic make-up of the western population relative to populations of bonobos throughout their range.

Wildlife Sanctuaries provide invaluable education for the local human population; especially for students, who represent the decision makers of the near future. ABC Bonobo Sanctuary welcomes over 8000 students per year (Andre 2001). It is hoped that these students experience empathy for human's closest relatives. It is certain they immediately discover the obvious similarities in behaviors and expressions of bonobos and humans. Even if only a small percentage of students actually act on their inspiration, the remaining students are likely to respond more in favor of the protection of nature, when faced with difficult decisions in later life.

The illegal bushmeat trade and illegal pet trade persist because the potential financial rewards far outweigh the risk of consequences. ABC sanctuary personnel regularly confiscate bonobo orphans at local markets, and it is hoped that dealers learn that business in pet trade is riskier and that they realize less profit. Wildlife sanctuaries provide a necessary infrastructure for the completion of the tasks involved with law enforcement. Without a safe location to deposit orphans, law enforcement would have extreme difficulty confiscating live bonobos, who require nourishment, supervision, and protection (Teleki 2001).

ABC Bonobo sanctuary provides the rehabilitation and the eventual re-introduction of wild born bonobos. It merits recognition and funding as an important source for the conservation of wild bonobos.

Implications for the Viability of the Western Population of Bonobos

Using the available information, a model of the expected HV1 region genetic make-up was developed for wild bonobos residing in the western area of distribution. This model is based on the assumption that records were accurate in reporting the regions of origin of the orphans. Though the sample size is small (N=16), leaving a large margin for sampling error, several factors increase the confidence of the likelihood that this sample can be used as a reliable initial assessment.

The most significant factor is geographical. The western population resides in the portion of the bonobo range that is closest to Kinshasa, where the ABC Orphanage is located. Bonobo orphans are fragile, malnourished, and traumatized by the massacre of their families. They often lack the will to survive (Andre personal communication). Poachers lack the knowledge of the care giving requirements needed. The majority of orphans are not likely to survive long journeys covering thousands of kilometers. These circumstances lead up to a conservative premise that ABC bonobos do have representatives from the western region.

The molecular evidence supports the assumption that these individuals could have come from a single population. Diversity measures are low (Table 10) relative to the other wild populations. If the majority of the individuals had come from dispersed

populations, the diversity levels would be expected to be higher, as they are with the whole ABC population.

ABC West diversity levels are lower ($\pi = 0.025$) , relative to the diversity levels of other populations (North: π=0.040; central: π= 0.032; south: π 0.030) and resemble the levels of the eastern population (π= 0.023), which is isolated from the other populations by over a thousand kilometers migrating distance (Eriksson et al. 2004). These values should be further investigated with more samples because of the possibility of sampling bias, due to small sample size (N=16).

Is lower diversity in ABC west a higher concern because of lack of matrilineal units in sampling? ABC bonobos were taken from the wild at different times and at different locations, and are therefore not likely closely related to one another. Mitochondrial DNA is inherited maternally, therefore all siblings will share the same mitochondrial DNA sequence. Sampling done from the same community could show sets of two to six identical genotypes due to association of maternal lines; estimated based on number of offspring potentially residing close to mother (Hohmann et al. 1999, Hohmann and Fruth 2002). Bonobos congregate in groups that consist of a mother, her offspring (females up to about age 11 and sons for life); other associated mothers with their offspring; and resident males (Thompson 1997, Kano 1992). Sampling done from the same community will likely show lowered overall diversity estimates and would not simply signal trends at the larger population level.

Because of the nature of their behavioral studies, both Hashimoto et al. (1996) and Gerloff et al. (1999) included mother-offspring groups in their sampling. Eriksson et

al. (2004) took precautions to avoid re-sampling the same individuals by sampling nests at a minimum of 5 meters apart, and groups at minimum of 10 kilometers apart. In addition, he sexed his samples and obtained microsatellite genotypes using 3 markers. Individuals of the same sex, same mtDNA genotypes, but different microsatellite genotypes were considered different individuals. However, he likely sampled several members of the same family.

As the viability of a population, measured by diversity indices, is usually estimated by comparing diversity levels of one population to those of another, the above methodology should remain useful. Similar methods are used in other primate studies, making the relative comparisons between species helpful. In this study, however, low diversity relative to groups sampled with the above methods, should be assessed with consideration of bonobo social and family structure.

This study did not provide enough evidence to substantiate the possibility that ABC West was recently genetically isolated from the southern and central populations. This is true especially in regards to the fact that ABC West shares four identical haplotypes with the central range. However, further genetic and field analysis should be carried out to supplement the current study, as ABC West did not provide the expected representation of haplotypes in a situation of unobstructed gene flow. Both the lack of haplotypes in the west from cluster 1 and the lack of shared haplotypes with the southern region leave some unanswered questions.

Is there gene flow back into the western range? With the apparent high levels of gene flow between the southern and central regions, if there is gene flow between the

central and west, why aren't representatives of cluster 1 or the southern region sampled in the west? Is this due to sampling bias, or could there be a lack of gene flow into the west? Further studies need to be done in order to answer this question definitively. If there is not gene flow back into the western range, the effects of stochastic events and the pressures of the bushmeat trade could render the population subject to a high risk of local extinction. In this case, the west would be prime location for focused protection. It is advisable to commit conservation efforts to protecting this region in order to maintain an intact ecosystem, in case of forest retraction (discussed below).

Implications About Current and Past Gene Flow of Wild Bonobos

Can historic migration routes be inferred from the distribution of haplotypes on the phylogenetic tree and median joining network? The addition of the ABC West sequences to the already existing pool of data on the HV1 region in bonobos illuminated some intriguing possible scenarios for ancient migration routes.

The neighbor joining tree and the median joining network, along with the geographical distribution of haplotypes, display geographically meaningful trends. When all haplotypes are considered in this study, along with estimated and known geographic origins, neither the Eastern haplotypes or ABC West haplotypes are represented in cluster one. All Eastern haplotypes and all ABC West haplotypes, except abcD (found in cluster two), are limited to cluster 3. As it had been expected that ABC West and the East would be more distantly related, given the geographic separation, questions arose as to why they

were both excluded from cluster three, which hosts haplotypes from all three other regions: South, Central, and North (Figures 10, 11).

All northern haplotypes are grouped in cluster 1; apart from EY1BH (with 10 individuals –14% of northern sample, found in cluster 3), and HI (with 1 bonobo, found in cluster 2). Southern and Central haplotypes are relatively evenly dispersed between clusters 1 and 3. According to the median joining network (Figure 11), haplotype S6abcAEY2NO (S6 from the south; and abcA from two bonobos with reported southern origins; EY2 and NO from the North, from 10 bonobos –17% of the northern sample) plays a central role in the distribution of haplotypes in cluster 1. It is the link to all other haplotypes found in cluster 1 (Figure 10).

Haplotype C4abcJK (C4 from the central region; and abcJK from both central and ABC West) plays a central role in cluster 3. It provides links to all other haplotypes in cluster 3. It also provides links to both of the other clusters (1 and 2) and represents the putative ancestral haplotype of all other haplotypes.

In consideration of this data set, the most parsimonious scenario for ancient bonobo migration patterns is that when a distant glacial event and drying of the forest occurred, bonobos retreated into wildlife refugia (Colyn et al. 1991) in the southwestern region; near Lakes Ntomba and Mai-Ndombe, and the confluence of the Congo, Sankuru, and Lukenie Rivers; where forest vegetation was sustained. In this population of ancient bonobos, the lineage C4abcJK was particularly successful.

When the equatorial forests became more expansive again, bonobos migrated into opening habitat. Some, especially close relatives of the C4abcJK lineage, remained in the

western region. One wave of immigration founded the Eastern region, and became subsequently isolated due to the increased flow of the Lomani River.

Another wave of migration, consisting of individuals with the haplotype S6abcAEY2NO, migrated north, and founded the northern lineages. When observing the median joining network, it is noteworthy that apart from two exceptions, all haplotypes with northern origins form terminal nodes. This observation indicates that central, west, and southern haplotypes provided the source for relatively newer northern haplotypes.

The anomaly of EY1BH occurring in cluster 3 could be explained by a second founding event. An ancestor of abcM (with reported central origins; from the northern part of central range (Figure 11) –three mutations from C4abcJK, could have migrated into the northern region; -after 4 more mutations; founding the EY1BH lineage. The central and southern populations maintained high gene flow between each other, creating a high diversity of haplotypes.

The value (t), calculated as the time to the most recent bottleneck (Rogers and Harpending 1992), dated to about 165,000 years ago. This date corresponds with a period of multiple expansions and contractions of the forest (Colyn et al. 1991). Since that time, many oscillations in the global climate have occurred, including a major glaciation event approximately twelve thousand years ago (Maley 2001).

According to the distribution of haplotypes on the geographic map, phylogenetic tree, and median joining network (Figures 5, 10,11), this initial speculation on ancient migration patterns of wild bonobos is plausible. The proposed migration pattern is not supported by standard statistical analyses. Tajima's D (Table 14) and mismatch

distribution (Figure 14), tests for evidence of a population bottleneck and expansion, were both not significant. The above proposed scenario suggests that the entire sample expanded from the C4abcJK lineage. Fu and Li's D and F statistics (Table 14) do not indicate that there is evidence of a higher than expected rate of recent mutations.

The statistical analyses and phylogenetic/geographic analyses do not concur with each other. However, one set of evidence should not be given precedence over the other in these early stages of analysis. All plausible scenarios for ancient and recent migration routes should be investigated further.

Understanding ancient migration routes allows the prediction of possible future migration routes due to global climate and anthropogenic changes. This can be used for planning corridors between protected areas and healthy habitat. If current warming trends were to lead to the eventual retraction of the current forest cover in the Congo Basin, this study would support the prediction that bonobos would benefit from a conserved forest corridor between the already established Salonga National Park in the central region (which holds the largest spread of haplotypic diversity (Eriksson et al. 2004) and the natural wildlife refugia in the Lac Ntomba and Lac Mai-Ndombe region ("west" in this study) (Figure 15).

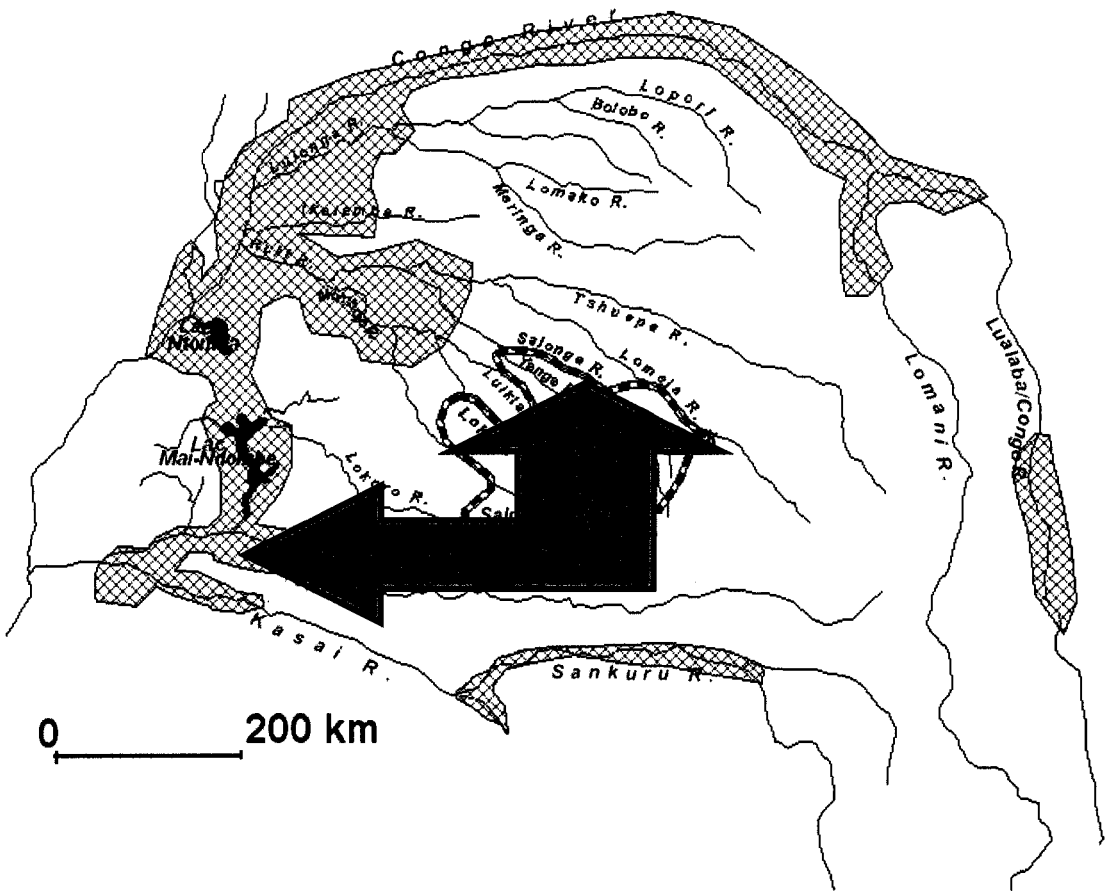

Figure 15. Forrest Corridor Recommended by this Study. Corridor is designed to preserve natural migration routes and maximum genetic diversity of bonobos and associated ecosystem. Pink arrow illustrates advised corridor. Shaded green areas represent Colyn et al.'s (1991) suggested natural forest refugia.

Implications for the Conservation of Bonobos

It was emphasized that current long term conservation projects should take into account past climatic, geologic, and ecological trends in order to maximize the long term viability of reserves (Hamilton et al. 2001). African rainforests have been less extensive and more fragmented throughout 80-90% of the past 800,000 years (Maley 2001). This suggests that less expansive rainforests have been the normal trend in Central Africa, throughout the majority of bonobo history.

With the compiling evidence that Earth is in a state of dramatic warming, the time for planning for the conservation of some of the last pristine forests is now. With current political stability in DRC restored, a window of opportunity for strategic planning of environmental protection is in place. The most effective results will come from direct involvement and education of the Congolese people (Thompson 1997).

The role that bonobos play in the forest ecosystems of the Congo has not yet been assessed. Studies on chimpanzees (Chapman and Onderdonk 1998, Sussman 1991, Wrangham et al. 1994, Balcomb et al. 2000) have suggested that primates may play a significant role in seed dispersal in the forest. Not only do they spread the seeds miles from the parent plants, studies have shown that the seeds have higher germination success after passing through the digestive system of large bodied mammals. With other wildlife also rapidly disappearing from the forests due to illegal poaching, basic systems that maintain balance could be threatened. More research needs to be done to understand the long term sustainability of forests becoming empty and the impact that the lack of bonobos would impose.

Being highly charismatic, the attention and funds that bonobos potentially attract for their own protection could benefit other wildlife species. Bonobos are the ideal candidate for a flagship species for DRC. In addition, the study of bonobos could be instrumental in understanding human evolution. Most models of human behavioral ancestry are based on data gathered from chimpanzees. Bonobos are equally related to humans as chimpanzees and demonstrate a large repertoire of unique behavior patterns, many of which are yet to be discovered. The fact that bonobos are little known to the general public could promote a sense of discovery for philanthropic donors, who could be instrumental in funding the conservation of wild bonobos and their ecosystems.

CONCLUSIONS: Evaluation of Project's Objectives and Hypotheses

This study was successful in reaching all three of its objectives.

First, an assessment is provided of the genetic structure of the HV1 region of bonobos with reported western origins. These results are used to discuss the projected viability of the wild western population. It is determined that the genetic variation in the western region shows evidence of low variability. This study recommends prompt further and continued sampling of the wild western population and a more extensive look at the genome, in order to substantiate the level of concern that these initial results report.

Second, this study provides a potential understanding of the ancient dispersal of the bonobo HV1 haplotypes. It suggests that haplotype C4abcJK could have served as the common ancestor of other observed bonobo HV1 haplotypes. Plotting the occurrence of this haplotype on a map along with all other observed haplotypes, in comparison to the phylogenetic and median joining distributions of haplotypes, along with hypothetical ancient wildlife refugia locations, revealed a potential ancient migration route.

Ironically, recent political instability in DRC has benefited bonobos to some extent. Logging concessions agreed upon before the war were put on hold (Baker et al. 2003), therefore expanses of bonobo habitat remain intact. With ensuing stability in DRC, logging concessions may become active again in the near future. This is the prime time to plan the protection of suitable expanses of habitat that are connected by wildlife corridors; allowing uninterrupted gene flow between populations. This study suggests that a corridor be established and protected between the Salonga National Park and the

region around Lake Mai-Ndombe and Lake Ntomba (figure 15). This initial genetic survey, combined with paleogeographical evidence indicate that this area provides a natural migration route that should remain intact in times of future climate shifts.

Third, this study evaluates the heterozygocity of the HV1 region in the ABC bonobo population. These results are discussed in terms of the expected viability of the ABC bonobos as a founding population for impending re-introduction into the wild. This study establishes that levels of variability of the HV1 region in ABC bonobos are comparable to the levels in established wild populations. This implies that there is no evidence for inbreeding concern. However, this study strongly asserts that the results of this study should not be considered as high priority in decisions regarding the re-introduction of bonobos to the wild, especially when considering the outcome of likely contact with wild populations. Further genetic analyses on nuclear DNA are highly recommended before the genetic component of re-introduction be given weight in the decision-making process. These results should be viewed as primary indicators, but not as conclusive evidence for the viability of the ABC bonobos as a founding population.

Based on the data gathered in this study, there was lack of support for this project's three stated hypotheses.

- Distinct mitochondrial haplotypes were not observed between regions.
- No evidence was found for haplotypes that were distinct enough to define regions, therefore it is not possible to assign regional origins using mitochondrial haplotypes.

- Mitochondrial variation in the HV1 region of ABC bonobos was comparable to the variation found in wild populations. The ABC population does not exhibit homogeneity.

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