Spring 2017

Yours, Mine and Ours: The Ethics of Using Human and Non-Human Primate DNA in Genetic Research

Brittany Truong

YOURS, MINE, AND OURS: THE ETHICS OF USING HUMAN AND NON-HUMAN PRIMATE DNA IN GENETIC RESEARCH

A thesis submitted to
Regis College
The Honors Program
in partial fulfillment of the requirements
for Graduation with Honors

by

Brittany Truong

May 2017

Thesis written by

Brittany Truong

Approved by

_____

Thesis Advisor

_____

Thesis Advisor

_____

Thesis Reader

Accepted by

_____

Director, University Honors Program

Table of Contents

List of Figures

List of Tables

Acknowledgements

First, I thank my thesis and research advisors, Dr. Marie-dominique Franco and Dr. Amy Schreier, for their faith, trust, support, encouragement, and guidance on not only this project, but also my other personal and academic endeavors. I am so honored to have been part of their research team. They have pushed me out of my comfort zone, taught me invaluable lessons and skills, challenged my thinking, and made me the scholar I am today. I will always treasure the time I spent training in the laboratory with Dr. Franco, the adventures I embarked on in the rainforest with Dr. Schreier, and the many laughs and meaningful talks I shared with both.

I would like to thank my thesis reader, Dr. Becky Vartabedian, for her thought-provoking questions and philosophical insight on this project. She opened my mind to new perspectives and helped me relieve some of my stubbornness, which have helped me grow as a person. I also thank my Honors Program advisors, Dr. Thomas Howe and Dr. Catherine Kleier, for their tough love, encouragement, and words of wisdom throughout my time at Regis University.

Thank you to my family and friends for their love, patience, understanding, kindness, concern, and support through this long journey. I would not have made it through with my sanity intact without them.

Finally, I would like to thank Regis University's Biology Department for laboratory space, materials, and equipment; Matt Barton, Nate Pryor, Heather Humphrey, Jibin Abraham, and Sarah Seiwald for their assistance in the laboratory; La Suerte

Biological Research Station for housing and an unforgettable field experience; and TriBeta National Biological Honor Society and the Regis University Research and Scholarship Council for funding.

I. Introduction

Growing up as a Vietnamese-American, I constantly wondered why I looked so different from strangers at the park or at Safeway. I encountered people with light and dark skin; blonde, red, and black hair; and blue, green, hazel, and brown eyes. In contrast, everyone in my family had distinct Asian features – tan skin, dark brown hair, and small, brown eyes. Even within my immediate family, nobody looked exactly alike, and in my mind, this was beautiful and miraculous. I was told that all humans were created in God's own image and likeness and this was supposedly the reason for our uniqueness. This answer never fully satisfied me, but I accepted it until the day I was first exposed to the complex study of genetics.

I later learned that DNA is the essence of our existence; indeed, it is the substance in our cells that distinguishes one individual from another. Approximately 99.9% of the human genome is shared within the entire human population, and the other 0.1% accounts for the genetic variation that is observed amongst individuals (National Human Genome Research Institute, 2016). There are only four nucleotide bases that make up DNA – adenine, thymine, cytosine, and guanine – and yet various sequences result in billions of different and unique characteristics. Nucleotides resemble the English alphabet. Twenty-six letters can be organized into a myriad of different words and sentences, and in the same way, four nucleotides are pieced together as unique DNA sequences. Together, they make one book, or genome, that is encrypted with information specific to one person. Sequences can differ by a single nucleotide or in the number of

copies of a specific sequence. These variations are called DNA polymorphisms, or DNA markers. Upon learning this, I quickly developed a passion for human genetics and vowed that I would one day pursue a career in it. I wanted to have a better understanding behind the mechanisms underlying our diversity.

In a genetics laboratory course during my sophomore year at Regis University, I had the opportunity to study a specific polymorphism in my own DNA. I extracted DNA from my cheek cells and used a process called polymerase chain reaction (PCR) to amplify a specific sequence, called the *Alu* polymorphism, at the PV92 locus on chromosome 16. This polymorphism is a 315 base pair long insertion that does not encode any proteins (Mighell, Markham, & Robinson, 1997). There are more than a million copies of it scattered throughout the genome. Many of these copies are considered "fixed," which means every person inherits a copy of the insertion at a specific locus. In contrast, at other loci, the *Alu* polymorphism is "non-fixed," and we can inherit zero, one, or two copies of it from our parents. An individual can be homozygous for the sequence (+/+), homozygous for its absence (-/-), or a heterozygote (+/-), depending on his/her heritage. At the PV92 locus, the frequency of the allele varies in different human populations across the world. For example, in France, the frequency is only 27.50%, which is significantly lower than Japan's frequency of 85.71% (Watkins et al., 2001).

In this laboratory activity, we were asked to predict our own genotype. We were given a blank world map, in which a small number of countries had been highlighted, and my classmates and I incorrectly assumed that the featured regions were the only areas where the *Alu* polymorphism was prevalent (Fig. 1). Based on this map, I predicted that I

would be heterozygous for *Alu*. I thought that I could have inherited one copy from my father's side because my great-grandfather is half Chinese, and China was one of the marked countries. On the other hand, my mother's family is only from Vietnam. Vietnam was not highlighted on the map, so I did not think I would have inherited the polymorphism from her side.

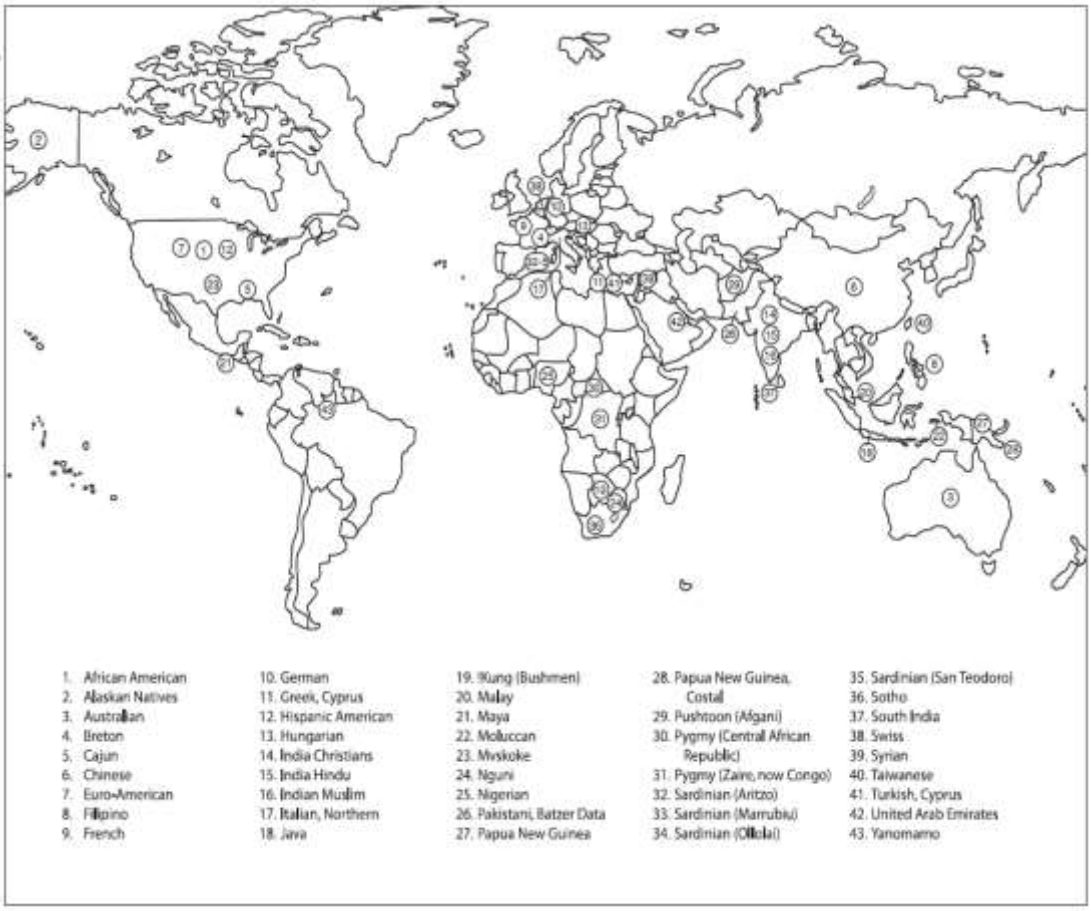

Figure. 1. World map that was provided in "Using an *Alu* Polymorphism to Study Human Populations" (Dolan DNA Learning Center, 2006), in which only certain countries are highlighted. These regions do not necessarily have a high or low insertion frequency. Rather, the laboratory manual simply asked students to focus primarily on these areas.

I found that I was homozygous (+/+) for the insertion. Still under the impression that the map depicted *Alu* frequencies, this discovery surprised both me and my family. For so many years, my mother's father, my grandfather, had told me that all of our ancestors originated from Vietnam, and he had always spoken with such assurance. The results of this simple genetic test suggested otherwise, and it made me seriously question my heritage.

I revisited the *Alu* polymorphism laboratory a few years later, only to realize that I had made a mistake in my prediction and interpretation. *Alu* at PV92 actually has a frequency of 87.50% in Vietnamese populations, not zero. In addition, China's allele frequency is 85.29% (Watkins et al., 2001). It was no wonder that I was homozygous for the insertion. This new discovery reaffirmed what I had always known about my family and my identity. Correcting the mistake did not significantly affect my life, but it was still a relief to know that my grandfather was not mistaken. A small misinterpretation gave me some emotional discomfort and had me living with the false impression that my mother's family was partially Chinese for years.

The full *Alu* polymorphism laboratory manual includes an "Informed Consent and Disclosure" page, but I do not remember ever seeing this page in my genetics laboratory. It states that students have the option to refrain from participating in the activity, and they should be willingly giving up their DNA samples, knowing that the results could reveal information about family relationships (Dolan DNA Learning Center, 2006). Had I known this, I could have chosen not to participate in the laboratory activity. I could have avoided the unexpected emotional discomfort I felt after seeing my results. In retrospect,

I probably would not have opted out of the activity because I loved extracting my own DNA and my discomfort was minor. Imagine, though, if we had been analyzing a region of my DNA that revealed something more significant, such as my likelihood of developing breast cancer. This bit of information would have surely given me severe emotional distress.

My experience has shown me the importance of informed consent when involving humans in research. Donors should be aware of how their DNA is being used and the potential risks. As a future genetic researcher, it is critical that I keep this in mind. Researchers are not always clear about how they will use genetic samples in their studies, which creates distrust in the general public (McGuire & Beskow, 2010). Many people are not willing to participate in genetic research because they are afraid that their personal information will be inappropriately shared with peers, employers, health insurance companies, or health care providers, leading to genetic discrimination (Genetics Home Reference, 2017). However, if we are to make any progress, we need DNA samples to study. In turn, researchers may feel the need to commit DNA theft. They can steal abandoned genetic samples, such as strands of hair left at the salon, and potentially invade a person's privacy rights (Joh, 2011). This then raises questions about DNA ownership and whether humans have rights to their genetic material and information.

Humans are fortunate in that they have the ability to voice their opinions and fight for the rights that they think they deserve. However, animals do not have this same liberty. Non-human primates, in particular, are regularly used as models for studying human genes and diseases because they are our closest relatives (NIH, 2007). Unlike

humans, they are not psychologically affected by the availability of genetic information, and they are not worried that their personal information will fall into the wrong hands; however, that does not mean we are free to extract, analyze, or modify their DNA as we please. In addition to the few million base pairs that humans and non-human primates share, we also share a capacity to suffer. Our shared sentience requires that we reevaluate non-primates' rights and avoid subjecting them to unnecessary suffering in genetic research.

I became interested in human and non-human primates' rights because of the research I had been conducting with Dr. Marie-dominique Franco and Dr. Amy Schreier. Since my junior year, I have been extracting DNA from and analyzing the genetic structure of two monkey species, mantled howler *(Alouatta palliata)* and white-faced capuchin *(Cebus capucinus)* monkeys, from La Suerte Biological Research Station (LSBRS) in Costa Rica. I worked with fecal samples that Dr. Franco and Dr. Schreier had collected during previous summers containing genetic material that was essentially stolen from the monkeys without their consent. During the summer of 2016, I traveled to Costa Rica to steal some more and then proceeded to extract information from the DNA once I returned to Regis University. I did not feel guilty doing either. I was not hurting the monkeys in any way. If anything, I was helping them by shedding light on how deforestation was negatively affecting their genetic diversity.

Like so many other people, I previously held an anthropocentric perspective on the world, and I valued human life over the lives of animals. I used to think that all research involving animals was justified if it provided some sort of benefit to humans,

and animals' rights were trivial compared to our own. I realize now, after working in the genetics laboratory, living with and developing such a strong connection to the monkeys at LSBRS, and writing my Honors Thesis, that humans and animals are so much more connected than I had initially realized. Both humans and animals have rights that must be respected when they are involved in any sort of research.

In Chapter II, I will discuss human DNA ownership and argue that while humans do not have rights to their genetic material, they should have ownership over their genetic information. Everyone has a right to decide whether or not he/she wants to know what information is contained within his/her DNA and who has access to it. Genetic research can move forward only if we protect individuals' rights. Then in Chapter III, I will discuss the ethical considerations of using non-human primates in genetic research. In addition to sharing over 90% of our DNA (NIH, 2007), non-human primates also share our capacity to suffer, and researchers should exercise the humane treatment principle when involving them in their studies. In Chapter IV, I will present my findings from the behavioral study I conducted in Costa Rica. I examined the effect of food availability on social structure and intraspecific interactions in the *A. palliata* population at LSBRS. I hypothesized that adult males and females would spend significantly more time with one another as opposed to with individuals of the same sex. This trend was observed amongst adult males, but there was no significant difference for females in the amount of time spent near individuals of the same or opposite sex. Finally, I will report my research on the genetic structure of *A. palliata* and *C. capucinus* at LSBRS. I rejected my first hypothesis that neither species would be in Hardy-Weinberg Equilibrium. Although both

species are inbred, my prediction that habitat fragmentation would more negatively affect *C. capucinus* compared to *A. palliata* is also rejected.

II. Human DNA ownership rights and the progression of genetic research

The completion of the Human Genome Project in 2003 led to rapid advancements in biotechnology, making it relatively quick, cheap, and easy to obtain and analyze genetic information (National Institutes of Health [NIH], 2016c). The Human Genome Project revealed the exact sequence of a person's DNA, which was a major step towards uncovering the function of specific genes and identifying mutations associated with genetic diseases; however, there is still much to be learned. Researchers require a supply of DNA samples to study, but many people are unwilling to donate samples of their genetic material (Presidential Commission for the Study of Bioethical Issues, 2012). They are uncomfortable with the thought of someone having direct access to their personal genetic information. Yet, humans discard DNA samples, such as hair or saliva, daily. Researchers could potentially steal and analyze abandoned DNA, but is it ethical to do so? Do humans have ownership rights to their DNA?

In this chapter, I discuss human DNA ownership rights and the implications they have for human genetic research. I argue that while humans do not own their genetic material once it has been removed from their bodies, they do have ownership rights to their genetic information. DNA contains very personal data, and we should decide who has access to it. The high demand for genetic samples in research can be met if researchers vow to respect our DNA ownership rights and are more transparent about how they will use our DNA in studies.

DNA is the essence of our existence. It is a string of four nucleotide bases – adenine, thymine, cytosine, and guanine – held together by a phosphate-sugar backbone and packed in the nuclei of our cells. These nucleotide bases can be arranged in an infinite number of ways. Just as the 26 letters of the alphabet are organized into words and sentences, nucleotides are pieced together to form genes. Together, they make one unique book, or genome, that is encrypted with information. Anyone can access a book in a library and gather information from it. Likewise, anyone can access another person's genome by taking a few cell samples and extracting the personal information found inside. Genetic researchers are particularly interested in studying these books to better understand human nature, such as our evolutionary history, our genetic variability, and the mechanisms of genetic diseases.

An entire genome is contained in the nucleus of each cell, and many people do not realize that they leave traces of their DNA everywhere they go. Strands of hair left behind at the salon or Starbucks coffee lids thrown in the trash have several copies of a person's genome that he/she has left behind. These are rather convenient sources of DNA for researchers to inconspicuously take and analyze without concerning the "donor" (Joh, 2011). This is called DNA theft, and though it is not so common today, there have been cases where researchers have profited from nonconsensual genetic sampling (Skloot, 2010).

Henrietta Lacks was an African-American woman who developed cervical cancer at the age of 31. She was diagnosed and treated by the doctors at John Hopkins Hospital in 1951. John Hopkins was known as a charity hospital and one of the only hospitals at

the time to treat African-Americans. Rebecca Skloot (2010), in her book *The Immortal Life of Henrietta Lacks*, describes:

> But first – though no one had told her that [the doctor] was collecting samples or asked if she wanted to be a donor – [he] picked up a sharp knife and shaved two dime-sized pieces of tissue from Henrietta's cervix: one from her tumor, and one from the healthy cervical tissue (p. 33).

This demonstrates how Lacks' cells were essentially stolen from her body without her knowledge or consent. Dr. George Gey and his research team immediately discovered that Lacks' cancerous cervix cells were immortal and could proliferate indefinitely with ample nutrients and space. Dr. Gey was the first to develop an immortal human cell line, which he called "HeLa" for the first two letters of the woman's first and last name. Then, in the 1950s, Microbiological Associates, a biological supply company, was the first to commercialize HeLa cells and make a profit by selling them to other laboratories (Skloot, 2010). Since then, many other biotechnology companies have cultivated HeLa cells and thrived off of them. The cell line is still currently used in research, and a single vial of HeLa cells costs over $400 (American Type Culture Collection [ATCC], 2017).

The exploitation of HeLa cells begs the question of whether it was ethical for the doctors to have actively taken tissue samples from Lacks without her knowledge and then make a profit from it (Skloot, 2010). They committed DNA theft and invaded her genetic privacy (Joh, 2011), but Skloot (2010) suggests that they did not do so out of ill will. They only wanted to study human cancer cells, and during this time period, it was not uncommon to take cells and overlook informed consent (Skloot, 2010). Moreover,

Tupasela (2011) argues that human samples, when it is no longer part of the person, becomes human waste because it "has no use value for its holder" (p. 514). We have no use for saliva left on a coffee mug or excised tissue samples. Aside from the fact that they hold copies of our genome, these samples have no value to us. We also leave our DNA everywhere, so it is not practical to initiate laws that criminalize DNA theft (Skene, 2005). Indeed, we do not have ownership rights over our genetic material after it has been removed from our bodies.

It is natural to feel uneasy about this. The thought of researchers stealing our DNA nonconsensually is unsettling; however, Skene (2005) points out that our discomfort stems not from an attachment to the genetic material, but rather, the information contained in it. Our DNA contains very personal data. Genes dictate our physical traits, such as eye color, height, and skin tone, that can be directly observed, but they can also reveal our likelihood of developing a fatal disease, which is more hidden. I concede that we have ownership rights to our genetic information because it contains sensitive data. Researchers must obtain consent from the donor before extracting the information and sharing it with others (McGuire & Beskow, 2010).

When we participate in genetic research, we have a right to decide whether we want to know the information contained in our DNA (Laurie, 1999; Andorno, 2004; Juth, 2014). Researchers often feel obligated to disclose significant findings to the person who donated their DNA, especially if they discover that he/she has a life-threatening condition (Hallowell, Hall, Alberg, & Zimmern, 2015). This is a sensitive situation. Finding out that we are at risk for or have developed a genetic disease can harm us psychologically

and emotionally, by causing "unwanted changes in self-image, reduced autonomy, [or] feelings of anxiety or depression" (Juth, 2014, p. 38). For example, patients who discovered they carried mutations in the *BRCA1* and/or *BRCA2* genes experienced elevated emotional distress thinking about possible future cancer treatments and their relatives' risks of developing cancer (Hamilton, Lobel, & Moyer, 2009). Some people prefer to remain in blissful ignorance and think genetic information is an unnecessary burden (Andorno, 2004; Herring & Foster, 2012). On the other hand, Takala (2001) dictates that we should know what is contained in our DNA because it can help us make more informed decisions about how we live. We can make simple lifestyle changes, such as changing our diets to avoid heart disease, but we can also be more drastic, like choosing not to have children so as not to pass on our condition and subject future generations to pain (Juth, 2014).

With genetic research, there is also concern that researchers may inappropriately disclose personal genetic information to other parties, such as employers, health care providers, health insurance companies, etc., leading to genetic discrimination (Genetics Home Reference, 2017). The National Partnership for Women & Families (2004) reports several cases where individuals have been inexplicably fired from a job or denied health insurance after individuals shared the results of their genetic tests. For example, a young boy with Long QT Syndrome, a rare genetic disorder that causes fast, chaotic heartbeats, was denied coverage under his father's health insurance because of his condition. In 2008, the United States issued the Genetic Information Nondiscrimination Act (GINA) to protect individuals from genetic biases. GINA prohibits health insurance companies from

using genetic testing to deny or underwrite coverage, and it prevents employers from using it to hire or fire employees (NIH, 2016a). However, GINA does not apply to companies that have less than 15 employees or to members of the United States Military. According to the NIH (2016a), GINA sets a "floor of minimum protection" and state legislators are responsible for issuing additional laws to protect individuals. Most states have passed laws that then regulate access to and the disclosure of genetic information (Presidential Commission for the Study of Bioethical Issues, 2012). Unfortunately, despite all of these laws seeking to protect individuals' DNA ownership rights, voluntary human involvement in genetic research is still low (Saulsberry & Terry, 2013).

Perhaps people would be more willing to participate if researchers were more transparent with their goals and the purpose of their experiments. Donors like to know how their DNA is being used, and this information should be provided in the consent forms they sign beforehand (Skloot, 2010). However, researchers often use broad blanket statements in these forms to give them greater flexibility (McGuire & Beskow, 2010). For example, in 2004, the Havasupai Native American Tribe filed a lawsuit against researchers at Arizona State University for using Havasupai blood samples in projects that tribe members had not agreed to (National Congress of American Indians, n.d.). The original study was designed to look only at the prevalence of type II diabetes in this community, but the samples were also used in schizophrenia and inbreeding studies (Markow et al., 1993). Inbreeding, in particular, is a taboo subject amongst the Havasupai people. Upon hearing that their samples were improperly used, they grew distressed and took the case to court. The tribe received a $700,000 settlement in 2010, and Arizona

State University returned all of the Havasupai DNA samples they had collected (National Congress of American Indians, n.d.). This demonstrates how important it is that researchers are explicit about how they intend to use collected DNA samples. They should not violate participants' trust. McGuire and Beskow (2010) wisely point out, though, that it is impossible to know upfront *exactly* how a sample will be used. Samples can be put aside and stored in biobanks for years. When researchers are finally ready for them, the original project that the donor agreed to participate in may have changed. It may be a hassle to contact the donor and inform him/her of experimental changes, but this step is crucial for maintaining trust between participants and researchers. Participants should also be comforted by the fact that by federal law, they can withdraw their consent to participate at any point, and researchers must stop using their samples (McGuire & Beskow, 2010).

Although there are many potential risks that come with taking part in genetic research, this should not discourage people from doing so. Rather, I think it is important that we do opt to participate when given the option. By 2015, over 1,000 human genomes were sequenced and made publicly available as part of the 1,000 Genomes Project (International Genome Sample Resource, 2015a). Anyone seeking to better understand human genetic variation can access these genomes. Researchers have already used this data and identified new genetic markers linked to medical conditions, including celiac disease, prostate cancer, glioma, type II diabetes, breast cancer, and diabetes (Zheng-Bradley & Flicek, 2016). This is thanks to the many people who have donated their DNA samples for genetic research. New data are currently being added to the public database

by the International Genome Sample Resource (IGSR) (International Sample Resource, 2015b). The IGSR is hoping to add samples from populations that were not part of the original 1,000 Genomes Project. This will allow researchers to develop an even better understanding of human genetic variation by including samples from across the globe; however, this can only be done if people willingly donate their DNA.

I have worked with DNA samples in the genetics laboratory since my junior year at Regis University, but I have never had to ask my research participants for their consent to be part of my study. This is because my study subjects have been mantled howler (*Alouatta palliata)* and white-faced capuchin *(Cebus capucinus)* monkeys who are not able to offer any form of consent (See Chapter III and V). In turn, I have been free to steal and analyze their DNA without worrying about genetic discrimination or causing them psychological harm. As a future genetic researcher, I am most interested in studying human diversity and the development of genetic diseases. I will soon be making the transition from studying monkeys to humans, and I will need to adjust my research practices accordingly. I recognize that I cannot force humans to participate in my future experiments by stealing their fecal samples, as I did with the monkeys. I must encourage them to do so by ensuring them that I will honor and respect their DNA ownership rights and by being transparent with what my studies entail.

III. Ethical considerations in non-human primate genetic research

In the previous chapter, I examined human DNA ownership rights and argued that humans have ownership of their genetic information but not necessarily the material after it has been removed from their bodies. Genetic studies involving humans are highly regulated in order to protect human interests and prevent physical or psychological harm. In contrast, the use of non-human primates in genetic research is still widely debated. There are studies that focus on the animals themselves, namely their evolution, kinship patterns, and diversity; however, because humans and non-human primates share over 90% of DNA, they are also useful models for studying human genes and diseases (National Institutes of Health [NIH], 2007). They offer many benefits in genetic studies, but it is important to keep in mind that humans and non-human primates share more than a few million DNA base pairs – we also share a capacity to suffer. This mutual sentience requires that we reevaluate how we conduct genetic research with non-human primates. Can we justify animal suffering if it provides a significant benefit to human life?

In this chapter, I discuss the ethical considerations that must be assessed when using non-human primates in genetic research. I argue that because both humans and non-human primates have the ability to suffer, researchers must apply the humane treatment principle when designing and conducting their studies. In other words, they must ensure that non-human primates are not enduring any unnecessary suffering during sample collection, sequence analysis, or genome modifications. As so, I have myself

implemented the humane treatment principle in my undergraduate research involving monkeys, and I plan on upholding this mindset as I pursue my career in genetic research.

I first became interested in these issues after having worked with monkey feces for over a year in the genetics laboratory at Regis University. Not all genetic research is conducted for the sake of humans. Some studies are designed to learn more about the animals themselves, namely their evolutionary history, kinship patterns, etc., which could have important implications for their well-being and existence. My research project was a conservation genetics project, part of larger study overseen by Dr. Marie-dominique Franco and Dr. Amy Schreier, in which I analyzed the genetic structures of mantled howler *(Alouatta palliata)* and white-faced capuchin *(Cebus capucinus*) monkeys from La Suerte Biological Research Station (LSBRS) in Costa Rica. I traveled to LSBRS to collect *A. palliata* and *C. capucinus* fecal samples in the summer of 2016, extracted DNA from the feces, and determined both species' genetic diversity by analyzing specific microsatellites (See Chapter V). To put it simply, I stole genetic samples from monkeys in Costa Rica and nonconsensually extracted information from their DNA. Although I discouraged this type of behavior in my previous chapter, my actions felt justified. Indeed, the primary goal of my research project was to determine if deforestation was negatively affecting genetic diversity in *A. palliata* and *C. capucinus* by forcing them to inbreed. I knew that my research was essentially helping the monkeys as my results will provide insight into their adverse situation. Moreover, none of my methods, from the sampling down to the genetic analysis, harmed the monkeys in any way.

Genetic samples can be obtained from animals noninvasively or invasively. In noninvasive sampling, Pauli, Whiteman, Riley, and Middleton (2009) explain, "Animals are unaware of sampling and, therefore, are unaffected by it…or animals are unrestrained and do not exhibit a chronic or severe stress response or experience reduction in survival or reproduction" (p. 350). For example, researchers can collect abandoned feces, hair, feathers, egg shells, and snake skins and extract DNA from these samples for their studies (Taberlet, Walts, & Lulkart, 1999). In contrast, invasive sampling consists of drawing blood or scraping tissues. Noninvasive sampling is attractive to many researchers, like myself, because it requires less equipment and minimizes physical pain. Moreover, projects that use noninvasive sampling are more likely to be approved by the Institutional Animal Care and Use Committee (IACUC). The purpose of the Committee is to minimize harm inflicted on research animals. This ensures that animals are not subjected to unnecessary suffering (Steneck, 1997).

Unfortunately, the quantity and quality of DNA extracted from noninvasive samples are much poorer compared to invasive ones, making them more difficult to analyze (Taberlet, Walts, & Lulkart, 1999). Dai, Lin, Fang, Zhou, and Chen (2015) extracted DNA from Chinese egrets *(Egretta eulophotes)* and found that blood yielded the highest DNA concentration (252.16 ± 17.05 ng/μL) when compared to plucked feathers (182.49 ± 7.95 ng/μL), shed feathers (13.59 ± 2.10 ng/μL), and feces (9.77 ± 1.83 ng/μL). In addition, noninvasively collected DNA tends to degrade readily and contains polymerase chain reaction (PCR) inhibitors, like melanin and keratin, which interfere with DNA amplification (McDonald & Griffith, 2011). In contrast, nucleated

blood is purer and more concentrated. For blood, DNA extraction only requires 50 μL of volume, and any remaining blood that is drawn can be preserved indefinitely. Should any problems arise, researchers can repeat the experiment using the excess. Those that collect samples noninvasively do not often have this same liberty, depending on how much of the original sample they were able to collect and how much was needed for DNA extraction. Researchers cannot make any mistakes in DNA extraction or amplification, nor can they repeat their experiments to validate their results when they have a small sample size. Rather than dealing with the many problems that come with analyzing noninvasive samples, many researchers opt for the more convenient route and study DNA collected invasively (McDonald & Griffith, 2011).

My own experience has shown me that it is incredibly difficult to work with and analyze DNA that has been collected using noninvasive techniques. The feces I analyzed had only small amounts of DNA. Extraction was difficult because the DNA was not evenly distributed in the stool, and the samples were often watery. I spent my first year of research trouble-shooting in the laboratory as I was unable to amplify DNA from *C. capucinus.* I had been using the same extraction and PCR procedures for both the *A. palliata* and *C. capucinus* samples. However, *C. capucinus* feces have even lower concentrations of DNA than *A. palliata,* and the DNA is more sensitive to time and temperature. Therefore, the samples had to be handled differently. After months of trial and error, I deteriorated my supply of feces, but I finally perfected the *C. capucinus* extraction and amplification protocols. Nevertheless, I knew that the DNA was fragile, and I worried that it might degrade if it was not properly stored or if it was left at room

temperature for too long. If the DNA had degraded, I would not have been able to redo some of the experiments because I exhausted my supply of original genetic material. Therefore, I sympathize with fellow researchers who work with noninvasive genetic samples.

Although I recognize how much easier it would have been to extract DNA from and analyze purer genetic samples, like blood, I also maintain that using feces was the more ethical route. Obtaining blood would have required capturing the monkeys, necessitating professional help to dart them with drugs (usually ketamine solutions) that temporarily immobilize them and cause them to fall from the trees (Jones & Bush, 1988). However, darting is a dangerous technique. Wasserman, Chapman, Milton, Goldberg, and Zigler (2013) found that darting adult red colobus monkeys *(Procolobus rufomitratus)* with telazol and ketamine led to an acute increase in cortisol levels, indicating physiological stress. Jones and Bush (1988) note that of the 27 redtail monkeys *(Cercopithecus ascanius)* they darted, two died and one was severely injured. In addition, because the understory of the forest was so thick, they could not predict where *C. ascanius* would fall and waited until they hit the ground to capture them (Jones & Bush, 1988). This demonstrates how darting is a dangerous and difficult procedure that inflicts unnecessary pain. Rather than risk harming *A. palliata* and *C. capucinus*, I chose to follow them in the rainforest and wait for them to defecate instead.

My research ethics have been guided by the "humane treatment principle" outlined by Gary L. Francione. He dictates, "[The] humane treatment principle…[is] the view that because animals can suffer, we have a moral obligation that we owe directly to

animals not to impose unnecessary suffering on them" (Francione, 2004, p. 113). The term "unnecessary" is key in Francione's definition, but it constitutes a gray area. How do we decide what is considered "unnecessary suffering," especially in genetic research involving non-human primates? There is a significant difference between simply analyzing sequences for the sake of the monkeys, as I have done, and analyzing and modifying the DNA to benefit humans. Cohen (2001) argues that we have a "duty" to use animals in biomedical experiments to promote human health and save lives (p. 5). In doing so, he implies that their suffering on our behalf is absolutely necessary. Because they are our closest evolutionary relatives, non-human primates are regularly used as models for studying human disease (Harding, 2013). Rhesus macaque *(Macaca mulatta)* oocytes have been injected with viruses expressing exon 1 of the human huntingtin (HTT) gene to model Huntington's disease. The authors argue that *M. mulatta* models more accurately mimic the neurological and behavioral mechanisms of Huntington's compared to rodent models (Yang et al., 2008). Researchers are drawn to non-human primates because they are often better models for studying human physiology, behavior, disease, and genetics (Harding, 2013). They do not see the experiments as "unnecessary suffering" because the monkeys are essentially helping humans.

Though this may be true, it is important to keep in mind that humans and non-human primates share more than physiological factors and homologous genes – we also share a capacity to suffer. Indeed, Ferdowsian et al. (2011) found that when chimpanzees *(Pan troglodytes)* were subjected to experimentation or experienced other traumatic events, they exhibited behaviors similar to those observed in humans with post-traumatic

stress disorder (PTSD) and depression. They refused to engage in play or grooming, were antagonistic towards peers, isolated themselves, and sat in a hunched, remote posture (Ferdowsian et al., 2011). Given our shared sentience, Francione (2004) supplements his humane treatment principle with the "principle of equal consideration," which states that we are required to "weigh our suffering in not using animals against animal interests in avoiding suffering" (p. 121). Before conducting an experiment, we must consider the costs and benefits to both parties, and the benefits to one must significantly outweigh the costs to the other (Sandoe & Holtug, 1996). However, in a primarily anthropocentric world as our own, we cannot help but prioritize our own needs above other animals'.

Francione (2004) has observed that even when animal and human interests have an equal weight, we almost always disregard the animals' interests in favor of our own. For example, *M. mulatta* have been used as models to test the efficacy of vaccines against *Shigella dysenteriae* 1 (SD1). SD1 causes Shigellosis exclusively in humans and non-human primates, and they experience the same symptoms, namely lymphocytosis, acute colitis, fevers, dehydration, and bloody diarrhea (Islam et al., 2014). However, only *M. mulatta*, not humans, are used in the initial stages of vaccine tests, demonstrating how we prioritize human interests above animals'. We tend to view animals as "property" in our society, or "nothing more than things" (Francione, 2004, p. 108). This type of mindset is alarming, as it gives researchers the false impression that they can use animals, particularly non-human primates, as they please. Therefore, instead of seeing them as "property," we should try to remember that non-human primates are our relatives.

That is not to say that we should completely ban the use of non-human primates in genetic research. Francione (2004) argues, "[We] ought to treat like cases alike unless there is a good reason not to do so" (p. 121). "Like cases," in my opinion, are the species that are most closely related to us, namely the great apes. Orangutans, gorillas, chimpanzees, and bonobos are our closest relatives, sharing between 97.0-98.8% of DNA with us (NIH, 2011; Wall, 2013). They have cognitive abilities and are capable of using tools (Chuecco, n.d.). In addition, *P. troglodytes* can communicate with humans through American Sign Language (Rivas, 2005) and even grieve deceased group members (van Leeuwen, Mulenga, Bodamer, & Cronin, 2016). These significant similarities render that they be treated like humans with regard to genetic research. They should be protected from experiments that inflict any physical, emotional, or psychological pain on them. The Great Ape Project, created in 1994, is working towards establishing international personhood rights to all of the great apes. Several countries, including Spain, New Zealand, and Austria, have already banned biomedical research involving great apes (Project R&R, 2017). In 2015, the United States banned the use of *P. troglodytes* in all biomedical research. *P. troglodytes* individuals owned by the NIH are expected to be reallocated to Chimp Haven, a federal sanctuary located in Louisiana, by 2025 – 10 years after the ban was instated (NIH, 2016b).

As we move along the phylogenetic tree, our relatedness to other non-human primates, such as monkeys including *M. mulatta*, *A. palliata,* and *C. capucinus*, decreases. In turn, they are no longer considered "like cases." In other words, our physiologic and genetic similarities are enough to justify their use in some research, but

not enough to offer them limited human rights, like the great apes. For example, humans share 98.8% of their DNA with *P. troglodytes* (Wall, 2013) and approximately 93% with *M. mulatta* (NIH, 2007). In addition, the great apes behave more similarly to humans than monkeys. Mark Mirror Tests were administered to *P. troglodytes* (Gallup, 1970), *M. mulatta* (Gallup, Wallnau, & Suarez, 1980), and brown capuchins *(Cebus apella)* (Roma et al., 2007), in which individuals were marked with a red mark on their heads and placed in front of a mirror. Only *P. troglodytes* noticed and reached for the mark on themselves, indicating that they are self-aware (Suddendorf & Butler, 2013).

Studying non-human primate DNA can, and has, provided incredible insight into our own genes and evolutionary history. Primates are thus indispensable in genetic research. Recent advancements in biotechnology, such as the development of the CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats) system, will undoubtedly increase their involvement in genetic research. CRISPR-Cas9 takes advantage of a mechanism utilized by bacteria to fight viral infections using a DNA-cleaving enzyme called Cas9 to edit genomes (Jinek et al., 2013). Chen et al. (2015) have mutated a dystrophin gene in *M. mulatta* to give them Duchenne muscular dystrophy (DMD), a severe genetic disorder characterized by progressive muscle loss. The genetically modified *M. mulatta* will allow researchers to study the pathology of the disease and uncover a future cure for humans. The significant benefits the study could provide justifies the research, though it pains me to think about *M. mulatta* being subjected to pain for our sake.

I have struggled thinking about where we should draw the line on what is considered morally ethical in genetic research involving non-human primates. It was not until just recently that I began advocating in favor of the humane treatment principle and the principle of ethical consideration. Before I traveled to Costa Rica and lived in the jungle for a month, I believed any kind of research involving animals was morally permissible if it provided any kind of benefit to human life because I value humans over animals. The gap that once separated humans from animals has decreased after my time with the monkeys and other wildlife in Costa Rica.

I developed a connection to them – a month in the rainforest will have that effect on you. I studied their social behavior (See Chapter IV) and observed firsthand how similar the monkeys were to us. Suddenly the line separating what is right and wrong for animal experimentation has blurred. I cannot even imagine the devastation I would feel if I heard about researchers genetically modifying *A. palliata* or *C. capucinus.* Before my trip, I would not have been bothered by it. It is sad to think that it took a month of being immersed in the rainforest for me to finally recognize that humans and animals, particularly non-human primates, have a deeper connection than I had recently thought. When we feel a connection to a particular animal, whether that is *C. capucinus* or simply a pet dog, we are more likely to fight against their mistreatment. It is important to recognize, however, that we are connected to *all* animals, not just our favorite ones. My willingness to allow researchers to modify *M. mulatta* on our behalf, but not *A. palliata* or *C. capucinus*, indicates that I am guilty of biases as well.

Of course, humans and animals share a right to live a life free from unnecessary suffering (Singer, 1975). Therefore, researchers have a responsibility to follow the humane treatment principle and the principle of ethical consideration when designing and conducting genetic experiments. Thus far, it has been relatively easy for me because I am simply *looking* at DNA sequences, not *modifying* it. Later in my career, I may be presented with opportunities to conduct more invasive genetic studies, like genome editing. I have already established a commitment to the humane treatment principle and principle of equal consideration that will guide my ethical decisions as I pursue a career in genetic research.

IV. The effect of food availability on intraspecific interactions in mantled howler monkeys *(Alouatta palliata)* in La Suerte, Costa Rica

Introduction

In the previous chapter, I discussed the ethical considerations that must be acknowledged when conducting research with non-human primates. I argued that researchers should not subject them to unnecessary suffering because they are sentient creatures like us. These feelings developed after I spent a month in Costa Rica observing mantled howler *(Alouatta palliata)* monkeys firsthand for this behavioral study.

Primate social systems are influenced by the environment, especially the availability of resources. Female primates organize themselves around the availability of food, and male relationships are structured around access to females (van Schaik, 1989). When food is abundant and evenly dispersed, contest competition, or direct aggression, between individuals is less likely to occur, and within group scramble competition, or the exploitation of resources, is more common (Koenig, 2002). For example, leaves are an abundant food source, so primates with folivorous diets typically have egalitarian social structures with nonlinear dominance hierarchies.

A. palliata are arboreal New World primates with a frugivorous and folivorous diet (Crockett & Eisenberg, 1987). Fruit is seasonal and not always readily available. Even when *A. palliata* are able to find fruit, they supplement their meal with leaves, indicating that they are more folivorous (Milton, 1981). Leaves are evenly distributed and

more accessible, so *A. palliata* are characterized as being non-aggressive and egalitarian with limited contest competition (Wang & Milton, 2002). Moreover, *A. palliata* groups are relatively large (>10 individuals) and contain multiple males and females (Clarke, Zucker, & Scott, 1986). Even so, direct social interactions between individuals are not common, and the easiest way to observe relationships in *A. palliata* groups is looking at proximity to an individual's nearest neighbor (Crockett & Eisenberg, 1987).

Males are not often in close proximity, but they are highly tolerant of one another. They generally benefit from living in multi-male groups. With more males, there are more individuals to help defend resources and protect other members (Bezanson, Garber, Murphy & Premo, 2008). *A. palliata* groups also consist of nonlinear hierarchies with one alpha, or dominant, male. The alpha male is one of the most vocal individuals and his position is usually spatially centered. Alpha males are prioritized and have greater access to food, but otherwise, all *A. palliata* males have equal access to estrous and receptive females (Wang & Milton, 2002). Nevertheless, reproductive success decreases when there are too many males in a single group (Ryan, Philip, Milton, & Getz, 2008). Males also rarely show aggression towards one another. When agonistic events occur, *A. palliata* males exhibit more "ritualized" behaviors, such as baring teeth or shaking branches, that do not require a high amount of energy (Jones, 1980). In contrast, it is not uncommon to see females close to one another. At Hacienda La Pacifica in Costa Rica, Zucker and Clarke (1998) found that high-ranking females were seen together more often than low-ranking ones, especially when one female had an infant. They also noted that when a new male immigrated into a group, females banded together and stayed in closer

proximity to one another for added protection. However, because their food is readily available, *A. palliata* females do not usually rely on one another for foraging and generally have weaker affiliative relationships compared to other primate species limited by a clumped distribution of food (Rodrigues, 2002; Wrangham, 1980).

Male-female relationships, or interactions between individuals of the opposite sex, appear to be common for *A. palliata.* Males usually prefer adult females over adult males or juveniles as their nearest neighbor (Wang & Milton, 2002; Rodrigues, 2002). Males spend more time resting and feeding close to females who act as potential mates. In addition, females develop relationships with males who can help them find food or provide protection from predators or infanticidal males (Bezanson et al., 2008). Although *A. palliata* are typically egalitarian, acts of aggression are often observed between males and females during feeding times (Wang & Milton, 2002).

This demonstrates how a specific primate social system has been shaped by its environment, especially the availability of food resources. Severe changes to the environment, such as habitat fragmentation, may have negative implications for primate social relationships (Arroyo-Rodriguez & Diaz, 2010). Since the 1970s, tropical rainforests of Costa Rica have been subjected to human-induced deforestation, leading to reduced habitats for inhabitants and a decrease in both the quantity and quality of food resources (Garber, Molina, & Molina, 2010; Arroyo-Rodriguez & Mandujano, 2006). La Suerte Biological Research Station (LSBRS) in Costa Rica is a fragmented forest and acts as a home for *A. palliata.* Because habitat fragmentation can have drastic negative effects on primate social systems, I have conducted a study to examine intraspecific

interactions among adult *A. palliata* at LSBRS. I predict that interactions between members of the opposite sex will occur more frequently than same-sex interactions in the *A. palliata* groups. More specifically, I expect an individual's nearest neighbor within 5 meters to be another individual of the opposite sex, and that behaviors, including grooming, mating, resting, feeding, following, and various forms of aggression will occur more frequently near and between individuals of the opposite sex. These behaviors are listed and defined in the provided ethogram (Table 1). Due to the even distribution of food, competition is not prevalent amongst females, leading to weak relationships and minimal interactions. Rather, females will associate more with males because they are dependent on them for protection. Males will then orient themselves around females for mating purposes, but they will limit aggressive interactions with other males as there is no need to compete with one another for estrous females.

Methods

Location of Study

I conducted this study from June 4-13, 2016 at La Suerte Biological Research Station (LSBRS) in Costa Rica (10°26'N, 83°46'W). LSBRS contains over 300 hectares (ha) of primary forest, secondary forest, and regenerating pastures (Garber et al., 2010). LSBRS is comprised of two forest fragments: the small forest and the large forest (Pruetz & Leasor, 2002).

Sampling Protocol

In order to examine the effect of an even food distribution on intraspecific interactions, I studied *A. palliata* for a total of 25 hours using instantaneous focal

sampling for 30 minutes with 2 minute intervals (Altmann, 1974). I collected 12.5 hours of data for adult males and 12.5 hours for adult females. At each interval, I noted the sex and age of the focal individual's nearest neighbor within a 5-meter radius (Wang & Milton, 2002). I also recorded behaviors including resting, feeding, and following and used ad libitum sampling to note instances of grooming, mating, and various forms of aggression (Table 1). For each 30-minute sample, the focal individual could not be out of view (OOV) for more than 10 minutes, and the same individual could not be re-sampled until at least 30 minutes had passed.

Table 1. Ethogram for *A. palliata*

Behavior	Code	Definition
Grooming	Gr	Using limbs to pluck at the hair of another individual.
Mating	Mat	Male individual mounts a female and thrusts visibly.
Resting	R	Little to no physical movement. Focal individual may move his/her head, limbs, or tail, but cannot travel more than 1 meter.
Feeding	Fed	Picking, chewing, and/or swallowing any type of food.
Following	Fol	Traveling in the same direction as and/or moving towards another individual. Focal individual may not be more than 2 meters from the nearest neighbor.
Aggression	Ag	Pushing, biting, grabbing, baring teeth at, and/or vocalizing towards another individual.
Other	Oth	Any other behavior that is observed.
Out of View	OOV	Focal Individual's behavior cannot be determined or the individual is not visible.

Data Analysis

I calculated the average percentage of time in which male and female mantled howler monkeys were within 5 meters of an individual of the same and opposite sex. I also examined the average frequencies of each behavior generally and then specifically calculated how often the behavior was performed around an individual of the same and

opposite sex. Finally, I analyzed the data using one-tailed two sample student's t-tests with a 0.05 significance level.

Results

For 25 hours, I observed intraspecific interactions in *A. palliata* populations by studying proximity to and interactions with and around an individual's nearest neighbor within 5 meters. On average, males spent significantly less time in close proximity to another male (3.86%) compared to in close proximity to females (34.33%) ($p<0.05$; Fig. 2). In contrast, there was no significant difference in the time females spent near males (16.22%) and females (17.63%) ($p>0.05$; Fig. 2).

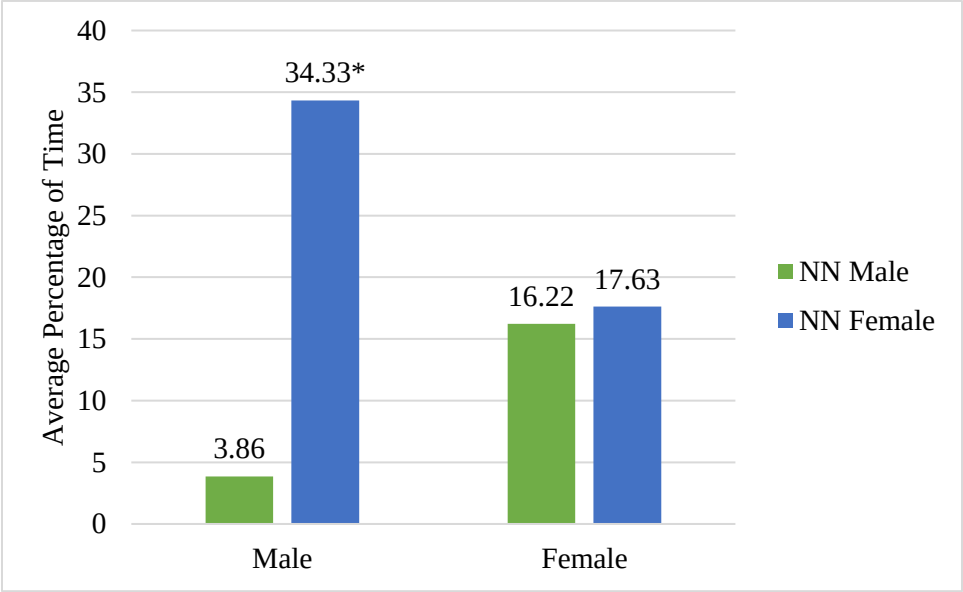

Figure 2. The average percentage of time A. palliata males and females spent with their nearest neighbor (NN) within 5 meters. The asterisk (*) indicates statistical significance at $\alpha = 0.05$.

Resting was the most common activity for both males (68.16%) and females (76.57%). Individuals rested alone, near immature monkeys, or near other adults. For males, only 1.9% of this time was spent around another male, which was significantly less than the time spent near a female (37.42%) ($p<0.05$; Fig. 3). In contrast, females rested near males 16.55% of the time and 18.72% near females, and this difference was not statistically significant ($p>0.05$; Fig. 3).

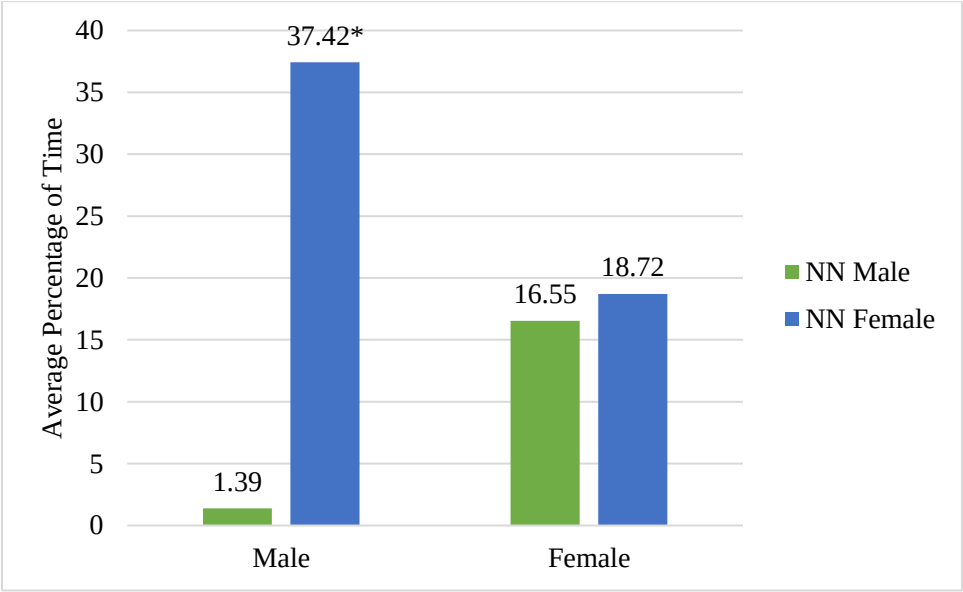

Figure 3. The average percentage of time *A. palliata* males and females spent resting within 5 meters of their nearest neighbor (NN). The asterisk (*) indicates statistical significance at $\alpha = 0.05$.

On average, males occupied 13.05% of their time feeding. Only 0.67% of the total feeding time was around another male, which was significantly lower than the amount of time spent in close proximity to a female (17.93%) ($p<0.05$; Fig. 4). Females similarly spent 13.53% of time feeding. However, there was no significant difference in the amount of time spent feeding near males (5.91%) and females (1.33%) ($p>0.05$; Fig. 4).

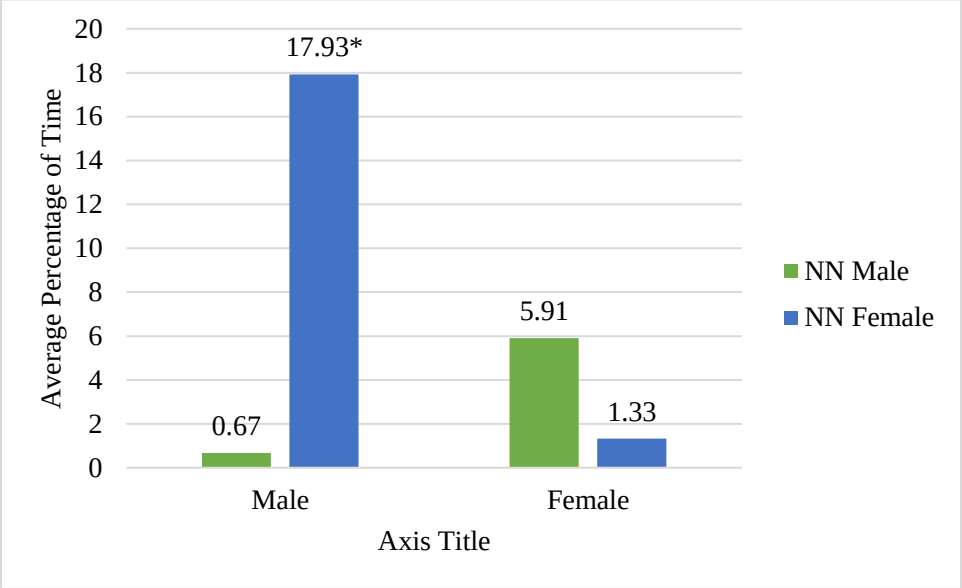

Figure 4. The average percentage of time *A. palliata* males and females spent feeding within 5 meters of their nearest neighbor (NN). The asterisk (*) indicates statistical significance at $\alpha = 0.05$.

Males occasionally followed close behind adult females, but only during 0.98% of their time. Conversely, females did not follow other individuals. There were two events of aggression. Both were performed by an adult male; however, one instance was directed at another male, while the other was towards an adult female. I did not observe any individuals grooming or mating with one another.

Discussion

To an extent, the data support my hypothesis that intraspecific interactions in *A. palliata* groups would occur more frequently between members of the opposite sex. Males spent almost 10 times as much time within 5 meters of a female than a male, and more than a third of their time spent resting was in close proximity to a female. Males rarely fed near one another and often chose to eat near females instead. This proportion of

the data supports my hypothesis that opposite sex interactions are more common in *A. palliata* groups. In addition, they were consistent with other studies which found that males prefer to be in close proximity to adult females, or individuals who can act as potential mates, rather than adult males or juveniles (Wang & Milton, 2002; Rodrigues, 2002). I only observed two events of aggression, one between a male and female and another between two males. Similarly, other studies have indicated that aggression events are rare because the even distribution of food restricts any need for competition (Jones, 1980; Koenig, 2002; Wang & Milton, 2002).

However, the data also reject my hypothesis. For females, there was no significant difference in the general time spent near males and females. Indeed, they spent an almost equal amount of time resting in close proximity to both. Females fed near males 5 times more often than near a female, but this difference was not statistically significant. Bezanson et al. (2008) point out that *A. palliata* male-female relationships are fairly common. Females rely on males to help find food or protect them, but they do not limit themselves to interacting only with members of the opposite sex. Likewise, Rodrigues (2002) suggests that females in close proximity to one another are probably kin. Nevertheless, the average proportion of time spent around another female was low, implying that the relationships between them are weak. With an even distribution of food, females are not dependent on one another for foraging and do not need to establish strong bonds (Wrangham, 1980).

It is important to note possible limitations of the study. It was often difficult to keep track of focal individuals, especially when the foliage blocked my line of sight

and/or multiple individuals were close together. My estimates for gauging 5 meters may not have been accurate, and an individual's nearest neighbor may have been hidden from view during sampling intervals. For many females, even though they were within 5 meters of an adult male or female, their nearest neighbor was deemed a juvenile because it was closest to them. Finally, because the sample size was small, the data may not accurately portray intraspecific interactions in *A. palliata* groups at LSBRS.

In the future, I would like to conduct a similar study looking at intraspecific interactions for white-faced capuchin monkeys *(Cebus capucinus)* at LSBRS. *A. palliata* and *C. capucinus* have different diets, which may lead to divergent social interactions. *C. capucinus* are mainly frugivores. Food is not readily available and is distributed in uneven clumps (Chapman, 1987). In turn, female-female interactions are more prevalent than male-male or male-female interactions because females rely on one another for finding and defending food (Fedigan, 1993; Perry, 1996). I can then compare the two species and further confirm the effect of food distribution on primate social systems.

V. Comparison of genetic structure between mantled howler *(Alouatta palliata)* and white-faced capuchin *(Cebus capucinus)* monkeys in La Suerte, Costa Rica

Introduction

In the previous chapter, I reported my findings from a behavioral study on mantled howler monkeys *(Alouatta palliata)* that I conducted at La Suerte Biological Research Station (LSBRS) in Costa Rica. I looked specifically at intraspecific interactions between adults and point out that primate social systems are shaped by the environment, especially the availability of resources. Severe changes to the environment, such as habitat fragmentation, can have important implications for not only primate social behavior, but also their genetic structures, as it can negatively affect their ability to disperse from their natal groups. Therefore, in this chapter, I present my genetic research analyzing genetic diversity and the level of inbreeding in *A. palliata* and white-faced capuchin monkeys *(Cebus capucinus).*

Some of the biggest threats to biodiversity over the last few decades are deforestation leading to habitat loss and fragmentation (Clarke, Zucker, & Scott, 1986; Arroyo-Rodriguez & Diaz, 2010). Forests are often cleared and then transformed for agricultural use. For example, in Costa Rica, a vast proportion of land now serves as banana and pineapple plantations (Garber, Molina, & Molina, 2010). Human-induced habitat destruction forces animals to abandon their native land and settle elsewhere. Moreover, the remaining suitable living spaces are now more scattered and have

decreased in size (Oklander, Kowalewski, & Corach, 2010). As a result, animals' access to resources is limited, placing certain species at risk for extinction.

For non-human primates, habitat fragmentation limits their ability to disperse (Arroyo-Rodriguez & Diaz, 2010). In most species of non-human primates, at least one sex emigrates from its natal group once it reaches sexual maturity. In doing so, males or females avoid competing with relatives for resources (Glander, 1992). Moreover, individuals who disperse can undergo random mating and thus avoid inbreeding (Storz, 1999; Oklander et al., 2010). Indeed, in continuous forests, genetic tests reveal that males and females within non-human primate groups are not as closely related compared to individuals in groups that reside in fragmented forests (Oklander et al., 2010). The latter is an indication that individuals are forced to mate within the same gene pool. Inbreeding limits gene flow, decreases heterozygosity, and negatively affects genetic diversity, ultimately resulting in species extinction (Pope, 1992).

Gene flow is the transfer of alleles from one population to another. Alleles are variations of a gene. In diploid species, such as *A. palliata* and *C. capucinus,* for each gene, individuals inherit two alleles, one from each parent. Consistent gene flow is necessary for increasing genetic diversity within groups. In diverse groups, there are individuals who have variations in their alleles that make them better equipped to survive a negative situation, such as the onset of a fatal disease. Those that survive can then pass on these beneficial alleles to future offspring. In contrast, in groups with low genetic diversity, a majority of group members are susceptible to the disease and will not survive (Oklander et al., 2010). Maintaining genetic diversity is important for the group's, and

ultimately the species', survival. Habitat fragmentation and deforestation act as major threats to genetic variation, particularly for non-human primates.

A. palliata and *C. capucinus* are New World monkeys with different diets, social behaviors, and dispersal patterns. *A. palliata* are folivorous, tree-dwelling monkeys. Both males (79%) and females (96%) disperse from their natal groups (Glander, 1992). Juveniles are forced out of their natal groups, and individuals may travel alone for up to 4 years before joining an already established group or creating a separate group with other emigrating individuals (Glander, 1992). When individuals disperse, they run the risk of predation or being rejected by groups that have already been established. In *A. palliata* groups the alpha male may not want to compete for food with new males attempting to join his group (Wang & Milton, 2002). Pope (1992) found that it is even more difficult for *A. palliata* females to join established groups, and they typically create new groups with other wandering females that have been forced to disperse. Although it is costly and dangerous to emigrate, both sexes in *A. palliata* do so to avoid inbreeding. *A. palliata* females reach sexual maturity at 4.5 years, and males remain in a single group for an average of 7.5 years. If the female matures before her father leaves the group, it is possible for incest to occur. Because most males and females disperse from their natal groups, however, incest is rare (Pope, 1992).

C. capucinus are also New World monkeys, but they are frugivores/insectivores and reside mainly in the understory layers of the forest (Garber and Rehg, 1999). Once they are 4 years old, *C. capucinus* males disperse, but females are philopatric (Perry, 1996; Jack & Fedigan, 2009; Wikberg et al., 2014). In other words, males will leave their

natal group, while females stay throughout their lives. Males often engage in parallel dispersal, meaning that they emigrate from their natal groups with a relative and join another group together (Wikberg et al., 2014). Because females are philopatric, they live in a group with other female relatives, with whom they develop strong affiliative bonds (Fedigan, 1993; Perry, 1996). In one study conducted in Santa Rosa, Costa Rica, a few *C. capucinus* females left their natal groups, but this is rare due to aggression imposed on emigrant females by outside groups (Jack & Fedigan, 2009). However, female philopatry increases the probability of inbreeding compared to when both sexes disperse. If only one sex disperses, then there is more limited gene flow in a group (Wikberg et al., 2014). Female *C. capucinus* reach sexual maturity at 6 years. On average, *C. capucinus* alpha males remain in a group for 4.5 years, but their tenure can last up to 15 years (Wikberg et al, 2017). Although incest is rare, Muniz et al. (2010) reported one incidence of incest and two cases where paternal half-siblings mated in Lomas Barbudal, Costa Rica.

Since the 1970s, deforestation has taken a major toll on the tropical rainforests of Costa Rica (Garber et al., 2010). A vast proportion of the land has been transformed for agricultural use, and much of the remaining forest is privately owned. Conservationists at La Suerte Biological Research Station (LSBRS) in Costa Rica are protecting the ecosystems by replanting trees, protecting the wildlife from hunters, and bridging the gap between privately owned forests and national forests by establishing biological corridors between them. LSBRS is home to both *A. palliata* and *C. capucinus*. Because habitat fragmentation can have drastic negative effects on the genetic diversity of organisms, I conducted a study to analyze the genetic structure of both the *A. palliata* and *C.*

capucinus populations using selected microsatellites. Microsatellites are short segments of DNA with 2-6 base pairs that repeat up to 80 times. These segments are highly variable amongst individuals and can be used as molecular markers to determine an individual's genotype and the genetic diversity of a given animal population (Winkler et al., 2004). Individuals have two alleles at each microsatellite locus. If the alleles are the same, the individual is homozygous, whereas two different alleles indicate a heterozygote.

Heterozygosity, or the proportion of heterozygous individuals at a locus, can be used to signify genetic diversity in a population. In addition, observed allele and genotype frequencies dictate whether or not a population is in Hardy-Weinberg Equilibrium. If the observed frequencies are consistent with the calculated expected values, then the population is not evolving. In other words, in the population, natural selection is not occurring; there are no mutations; individuals are randomly mating; the population is large enough (Andrews, 2010). I estimated genetic diversity and inbreeding using F-statistics (Wright, 1951). Using observed and expected heterozygosity frequencies, I calculated an inbreeding coefficient, F_{IS}, for each species. F_{IS} measures any changes in heterozygosity due to non-random mating in a subpopulation. Values range from -1 to +1, where -1 indicates excess heterozygosity, while +1 implies excess homozygosity.

For my study, I compare the genetic structures of *A. palliata* and *C. capucinus* populations and analyze the effects of human-induced habitat fragmentation on their genes. I hypothesize that neither species will be in Hardy-Weinberg Equilibrium and that

evolution is occurring in both. There will be reduced gene flow and a high level of inbreeding for both species because habitat fragmentation limits the monkeys' ability to disperse. However, due to their differences in dispersal patterns, I predict that the genetic structure of *C. capucinus* will be more negatively affected than that of *A. palliata* because only one sex of *C. capucinus* disperses from the natal group. *C. capucinus* will be more susceptible to inbreeding and have a more positive F_{IS} value than *A. palliata.*

Methods

Location of Study

I collected fecal samples for my study at La Suerte Biological Research Station (LSBRS) in Costa Rica. Additional information about LSBRS can be found in Chapter IV under the Methods section.

Fecal Sample Collection

In order to study the genetic structure of *A. palliata* and *C. capucinus* monkeys, I collected fecal samples from individuals of both species as a non-invasive DNA collection method since fecal samples have epithelial cells from the monkeys' gastro-intestinal linings. I, along with other students and professors, collected fecal samples from the forest floor after monkey defecation events using sterile tongue depressors. For each fecal sample, I transferred approximately 5 g of fresh feces into 5-ml collection vials containing 2.5 mL of RNA*later*™ Stabilizing Solution (Thermo Fischer Scientific), labeled the vials (i.e., date, sex, age-class), and stored them at room temperature at LSBRS. At the end of the field season, the samples were shipped via FedEx to the Biology Department at Regis University, where they were stored at -20^{o}C for processing.

DNA Extraction

I extracted all DNA from gastro-intestinal epithelial cells found in the fecal samples using the QIAamp DNA Stool Mini Kit (Qiagen) and a modified protocol. For both the *A. palliata* and *C. capucinus* samples, I lysed 1 g of stool in 5 mL of InhibitEx buffer, rather than 10 mL (as indicated by the manufacturer) in order to increase the concentration of purified DNA. In *C. capucinus* samples, I incubated the samples in Buffer AL at room temperature for 24 hours, and the final elution with Buffer ATE incubated at room temperature for 30 minutes before centrifuging at full speed (Hiramatsu et al., 2005). There were 25 samples of purified DNA for each species, and all samples were stored at -20^{o}C.

Nuclear Microsatellite Genotypes

Polymerase Chain Reaction and Sequencing. I used a process called polymerase chain reaction (PCR) to amplify polymorphic microsatellites found in the DNA. I amplified *A. palliata* sequences using 0.5 µM of forward and reverse primers Apm01 for *A. palliata* (Cortés-Ortiz, Mondragón, & Cabotage, 2009) and *C. capucinus* sequences using 0.46 µM of forward and reverse primers Ceb02 (Muniz & Vigilant, 2008). PCR reactions contained 1X Taq polymerase buffer (New England BioLabs), 1.5 mM $MgCl_2$ (Thermo Fischer Scientific), 200 µM dNTPs (Qiagen), forward and reverse primers, and 1.5 units of Taq DNA polymerase (New England BioLabs). I added approximately 50 ng of DNA to each reaction, and used sterilized RNAse free water to bring the final volume to 20 µL. For *A. palliata* samples, PCR cycles were as follows: initial denaturation at 94^{o}C for 2 minutes; 35 cycles of: denaturation at 94^{o}C for 20

seconds, annealing at 64ºC for 20 seconds (Cortés-Ortiz et al., 2009), extension at 72ºC for 45 seconds; and a final extension at 72ºC for 10 minutes (Nechvatal et al., 2008). For *C. capucinus* samples, PCR cycles were as follows: initial denaturation at 95ºC for 3 minutes; 45 cycles of: denaturation at 95ºC for 30 seconds, annealing at 55ºC for 30 seconds, extension at 72ºC for 30 seconds; and a final extension at 72ºC for 30 minutes (Muniz & Vigilant, 2008).

To ensure that the sequences were amplified successfully, I identified the PCR products using 1.75% agarose gel electrophoresis run at 100 V for 1 hour. I used Bullseye DNA SafeStain (MIDSCI) and an ultraviolet lamp to visualize the PCR amplification products, and I photographed the gels.

After ensuring the presence of the various microsatellite sequences in each sample, I repeated the PCR reactions and sent 20 µL of the crude PCR products and 10 µL of the corresponding forward primer to Functional Biosciences for Sanger DNA sequencing (Madison, WI). Once I received sequencing results, I analyzed the electropherograms using Sequencing Scanner Software 2.0.

Allele Typing

The sequencing data allowed me to better analyze the genetic structure of the two monkey species. I was able to genotype the individuals and determine whether the monkeys were heterozygous or homozygous for a specific microsatellite. I devised a list of rules for analyzing the nucleotides in the electropherogram (adapted from DNA Sequencing Core, 2016; Barton, 2016).

1. I looked at how clean the beginning of the sequence was before the repeat motif. If the sequence had many overlapping peaks, then I determined this was a heterozygote and proceeded to count the number of repeats for the motif.
2. If the sequence was clean, then I started base counting using the first clear occurrence of the repeat motif.
3. The last distinct base of the repeat motif marked the end of the length of the first allele. Towards the end of the sequence, the peaks had more overlaps. These overlaps were equal in height, but half as tall as the repeat motif that preceded them. If there were additional repeat motifs after the last distinct base, then this was a heterozygote, and I proceeded to count the bases for the second allele (Fig. 2B).
4. If there were no additional repeat motifs after the last distinct base, then I determined this sample to be homozygous (Fig 2A).

Statistical Analysis

I analyzed the data by first determining the allele frequencies. I tested the null hypothesis of no evolution using Hardy-Weinberg Equilibrium and tested the significance between the observed and expected genotypes using a chi-square goodness of fit test. The null hypothesis was rejected when the p-value from the chi-square test was less than 0.05. These allowed me to indirectly assess the level of inbreeding in the population. Then I calculated the observed (H_o) and expected (H_e) heterozygosities and used these to determine the inbreeding coefficient (F_{IS}) for each species using the formulae outlined in Peakall and Sound (2009) and Barton (2016) (See Box 1). These allowed me to directly

assess the level of inbreeding within and between various groups of individuals (Oklander et al., 2010).

Box 1: Heterozygosity measurements and fixation index. The two species considered in this study are *A. palliata* and *C. capucinus*. Values for F_{IS} range from -1 to +1. Values close to zero indicate non-random mating. Positive values indicate inbreeding and excess homozygosity, while negative values indicate excess heterozygosity.

H_O is observed heterozygosity in each species and is given as the proportion of *N* samples that are heterozygous at the locus.

$$H_O = \frac{No.of\ Heterozygotes}{N}$$

H_E is expected heterozygosity in each species and is the sum of the squares of allele frequencies subtracted from 1.

$$H_E = 1 - \sum p_i^2$$

F_{IS} is the inbreeding coefficient within individuals relative to the population, measuring the reduction in heterozygosity of an individual due to non-random mating within its subpopulation.

$$F_{IS} = \frac{H_E - H_O}{H_E}$$

Results

To begin analyzing the genetic structures of the *A. palliata* and *C. capucinus* populations, I verified that the DNA was extracted from the fecal samples and contained the various microsatellite loci using PCR and gel electrophoresis. The gel was insufficient for genotyping individuals, but it confirmed amplification of the microsatellites. At the Apm01 locus, alleles were between 208-220 base pairs long (Fig. 5A) (Cortés-Ortiz et al., 2009), and at the Ceb02 locus, alleles were between 225-233 base pairs (Fig. 5B) (Muniz & Vigilant, 2008). Sanger sequencing allowed me to more closely analyze the microsatellites. Using the electropherograms generated for each sample, I counted the sequence repeats and determined the individuals' genotypes (Fig. 6A & 6B). I found five different alleles at the Apm01 locus, and each allele ranged from 42-50 base pairs. I also observed only two alleles at Ceb02 that were either 32 or 36 base pairs long. I calculated the allele frequencies at the Apm01 and Ceb02 loci, and using chi-square goodness of fit tests, I found that both species were in Hardy-Weinberg Equilibrium. There was no significant difference between the observed and expected allele frequencies in *A. palliata* [χ^2 (2, N =25) = 19.61, $p > 0.05$] and *C. capucinus* [χ^2 (2, N =25) = 0.66, $p > 0.05$].

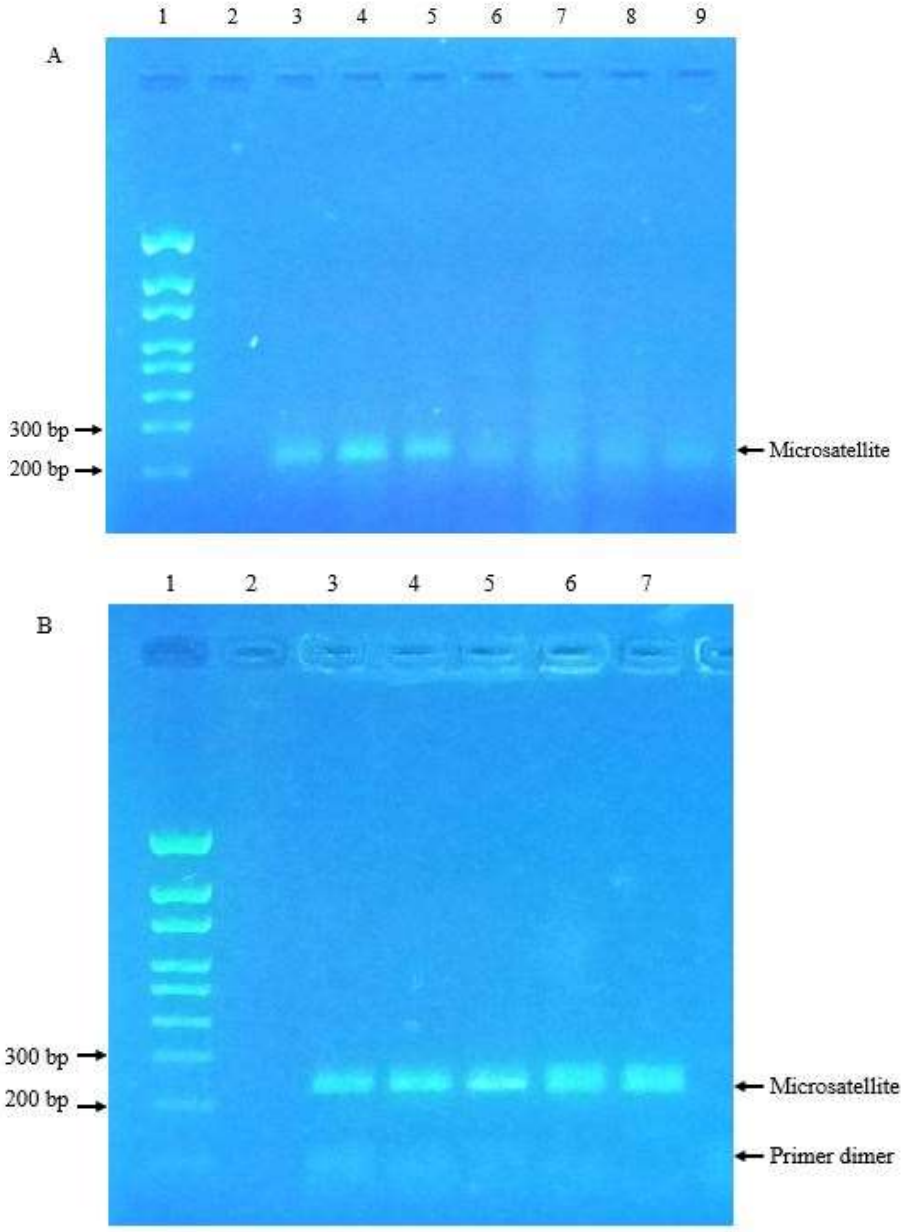

Figure 5. Gel electrophoresis results following PCR. (A) *A. palliata* samples (lanes 3-9) amplified with primer Apm01. The microsatellite bands are between 200-300 base pairs, which was expected (Cortés-Ortiz et al., 2009). (B) *C. capucinus* samples (lanes 3-7) amplified with primer Ceb02. The microsatellite bands are between 200-300 base pairs, which was also expected (Muniz & Vigilant, 2008). The primer dimers at the bottom of the gel formed because the primers annealed to one another, instead of the template DNA.

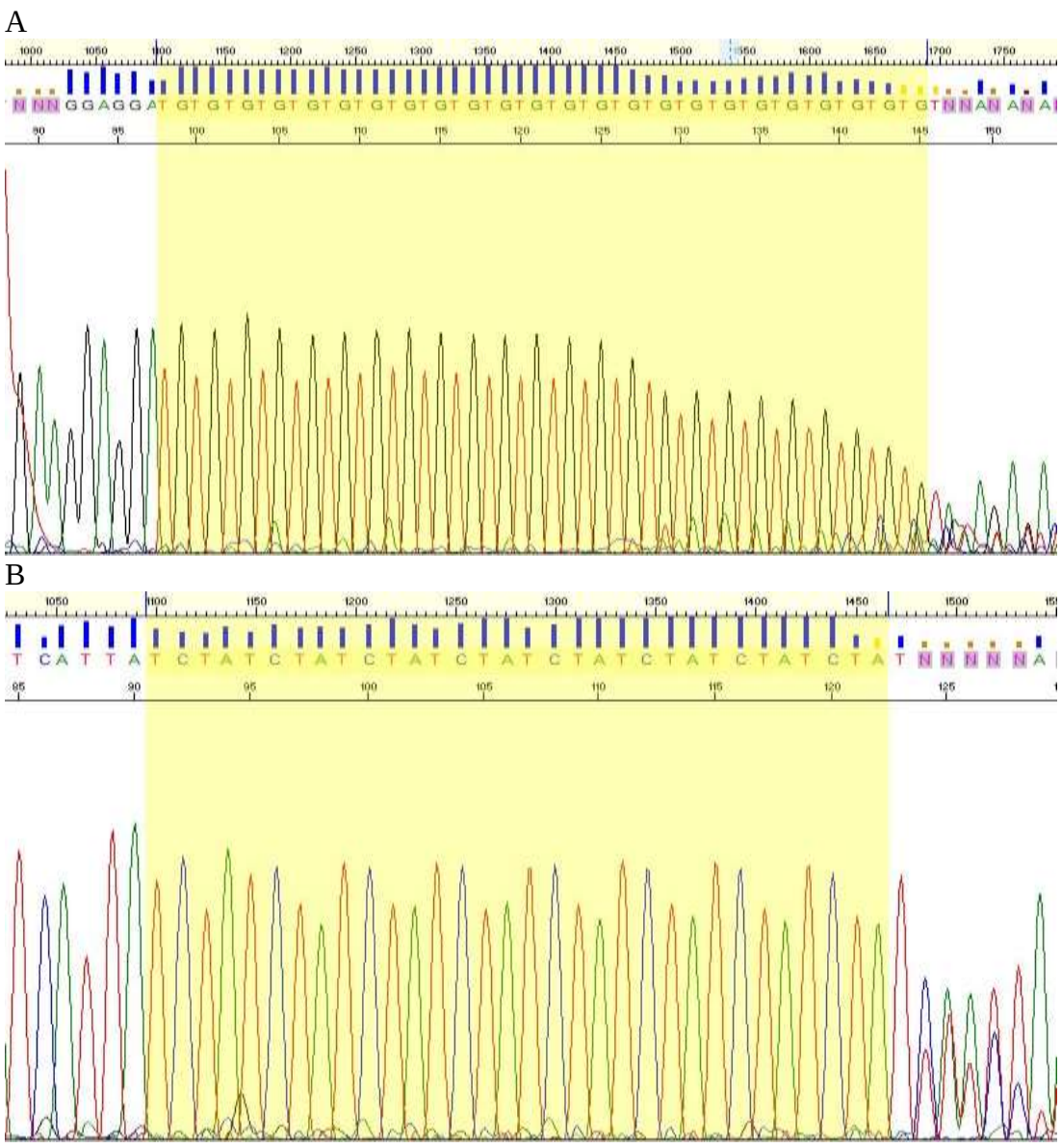

Figure 6. Electropherogram results from Sanger sequencing. (A) *A. palliata* sample amplified with primer Apm01. The highlighted yellow shows the TG repeats in the microsatellite. This individual is homozygous with 24 repeats on both alleles. Following the last TG, the sequence is TA. (B) *C. capucinus* sample amplified with primer Ceb02. The highlighted yellow shows the TCTA repeats in the microsatellite. The individual is heterozygous with 8 repeats on one allele and 9 on the other. After the last TCTA, past the yellow highlight, the peaks are overlapping, have equal heights, but are about half the height of previous peaks.

For both *A. palliata* and *C. capucinus*, there were more homozygote individuals than heterozygotes. At the Apm01 locus, 17 of the 25 samples were homozygous, and similarly, 18 of the 25 *C. capucinus* were also homozygous. I used F-statistics to estimate the observed (H_O) and expected (H_E) heterozygosities in each species. In *A. palliata,* the H_O was 0.320, and this was less than the H_E of 0.449. For *C. capucinus*, the H_O was 0.280, which was only slightly greater than the H_E of 0.241 (Table 2). Using these values, I calculated the inbreeding coefficient (F_{IS}) in each population. In *A. palliata*, F_{IS} was positive at 0.287, indicating that the population is inbred. However, *C. capucinus* had a negative value of -0.163, which implies that the population was more heterozygous and not as inbred (Table 2).

Table 2. Observed (H_O) and expected (H_E) mean heterozygosities and inbreeding coefficients (F_{IS}) for *A. palliata* and *C. capucinus.*

Species	H_O	H_E	F_{IS}
A. palliata	0.320	0.449	0.287
C. capucinus	0.280	0.241	-0.163

Discussion

The data reject my hypothesis that neither *A. palliata* nor *C. capucinus* would be in Hardy-Weinberg Equilibrium. The observed allele frequencies in both species were consistent with the calculated expected values. Although the H_O of *A. palliata* was lower than expected, this value was not statistically significant ($p>0.05$). The H_O value in *C. capucinus* was actually greater than the H_E, which implies the population is more heterozygous. Because both species are in Hardy-Weinberg Equilibrium, evolution is not occurring. This means neither are experiencing a significant amount of genetic pressure --

natural selection is not occurring; there are no mutations; there is gene flow; and individuals are randomly mating (Andrews, 2010).

To an extent, my results support my hypothesis that both species are inbred. The positive F_{IS} value for *A. palliata* implies excess homozygosity in the population. Although the F_{IS} in *C. capucinus* was negative (-0.163, Table 1), the value is closer to zero than -1. This suggests that the population is heterozygous, but not excessively so. Pope (1992) points out, though, that F_{IS} values do not always accurately represent inbreeding in a population, at least not initially. In her study, she found that low migration rates in red howler monkeys *(Alouatta seniculus)* led to high levels of inbreeding in the whole population, but she calculated negative F_{IS} values, or high heterozygosity within individual groups. The F_{IS} value should match what is occurring in the population and will probably become more positive over time (Pope, 1992). Unfortunately, to my knowledge, there have been no other studies that calculated F_{IS} values for *A. palliata* and *C. capucinus* at LSBRS. Therefore, I do not know how the F_{IS} values have changed as a result of deforestation. My study can act as a baseline for future genetic studies conducted at LSBRS.

Next, I reject my primary hypothesis that *C. capucinus* would be more inbred than *A. palliata.* My results are consistent with other studies that support this finding. Muniz et al. (2006) report that although only male *C. capucinus* disperse from their natal groups, there is almost no inbreeding in *C. capucinus* populations. Alpha males can remain in a group for up to 15 years and are the fathers of most offspring in a group.Muniz et al. (2010) assert that alpha males are willing to share estrous females with subordinate males

that they have strong bonds with. Wikberg et al. (2017) assert that *C. capucinus* females actively avoid inbreeding and are more inclined to do so because they are more invested in their offspring than males. Females also prefer to mate with strong, young males that recently emigrated into their group (Wikberg et al., 2017). These studies did not look specifically at the effect of habitat fragmentation on inbreeding and dispersal in *C. capucinus*; however, they suggest that inbreeding would still be rare because individuals very actively avoid inbreeding when mating.

A. palliata, in contrast, do not. Their primary strategy for avoiding inbreeding is forcing both sexes to disperse from their natal groups (Glander, 1992). I found that the *A. palliata* population at LSBRS was inbred. It is likely that habitat fragmentation negatively affected their ability to disperse from their natal groups. It is dangerous and costly to disperse, but it is necessary if individuals want to avoid inbreeding and competing with relatives for resources (Glander, 1992). Oklander et al. (2010) found that in continuous forests, black-and-gold howler *(Alouatta caraya)* monkeys emigrated more easily in continuous forests, and groups consisted of individuals that were not closely related. In fragmented forest groups, many females chose not to disperse and genetic relatedness was higher compared to continuous forest groups (Oklander et al., 2010; van Belle, Estrada, Strier, and Di Piore, 2012).

Because *A. palliata* are not as proactive in inbreeding avoidance as *C. capucinus*, it makes sense that *A. palliata* are more vulnerable to habitat fragmentation at LSBRS. Clarke, Collins, and Zucker (2002) argue that *A. palliata* are behaviorally flexible and do adjust to environmental changes over time. In their study conducted in 1984-2000, *A.*

palliata at Hacienda La Pacifica, Costa Rica responded to deforestation by establishing longer, more narrow home ranges and extending the length of daily travel. Still, their home ranges are only about 40 ha (Neville, Glander, Brata, & Rylands, 1988), which is substantially less than *C. capucinus'* home range of 116 ha (Crofoot, 2007). In addition, as an arboreal monkey, *A. palliata* is found almost exclusively in the trees (Crockett & Eisenberg, 1987). *C. capucinus* are largely arboreal as well. However, they also spend time traveling on the ground, and they may do so to move between forest fragments (Gebo, 1992). As a result, *C. capucinus* are more likely to encounter other groups which encourages gene flow in the population. As co-inhabitants of the fragmented forests of LSBRS, *A. palliata* and *C. capucinus* are exposed to the same environmental pressures, but their behavioral differences render *C. capucinus* better at maintaining its genetic diversity than *A. palliata.*

It is important to note the limitations to this study that may have affected my results. The sample size was small at only 25 for each species. In *A. palliata,* 11 of these samples were collected from individuals in the small forest fragment, while 8 *C. capucinus* samples were from this part of LSBRS. The small forest is approximately 15 ha, which is half the size of the large forest. There are fewer monkey groups living in the small forest and gene flow is more limited (Pruetz & Leasor, 2002). This could have contributed to the observation that *A. palliata* was more inbred. For this study, I looked at the inbreeding within the whole population. It would have been interesting to look at the level of inbreeding within individual groups. In addition, due to time constraints and technical difficulties, I only analyzed one microsatellite for each species. The data would

have been more complete had I been able to analyze more loci, as heterozygosity in microsatellites do vary. More data would have allowed me to more accurately judge whether or not the species were inbred. Finally, there may have been errors in my allele scoring. It was difficult to analyze the electropherograms and count the number of repeats for each allele, especially when there were many peaks overlapping one another. This could have led to errors in genotyping.

This study has demonstrated how human-induced habitat fragmentation is negatively affecting the genetic structures of these two monkey species. It will act as a genetic baseline for evaluating the effectiveness of conservation projects at LSBRS, including the construction of biological corridors between the forest fragments that will allow organisms to move more freely between them (Garber et al., 2010). Humans consume much of earth's resources and destroy habitats without considering how this may affect the environment or its inhabitants. As stewards of the earth, we all have a responsibility to care for the environment and ensure that it is well-maintained for future generations. This includes both humans and non-human species.

VI. Conclusion

When I look back on my time at Regis University, it is amazing to see how much I have grown as a student, researcher, and person. This personal growth began the moment I signed on to be part of Dr. Marie-dominique Franco and Dr. Amy Schreier's research team during my junior year. I was part of every step in the research process, from the fecal sample collection in the rainforests of Costa Rica, down to the genetic analysis in the laboratories in Denver, CO. I never thought that working with monkey feces could have affected my life in such a positive way.

Like so many others, I used to have a primarily anthropocentric viewpoint on the world, and I was not concerned with animal rights or environmentalism. I knew these were important issues because I had learned about them in classes, but I did not feel truly invested in them. I had always prioritized our human wants and needs over those of animals or the environment. For example, I believed that any kind of research involving animals was morally permissible if it provided any kind of benefit to human life. I also thought sustainable practices, such as recycling, were an unnecessary hassle. It was not until after I had spent a month in the rainforest; walked the barren paths between the forest fragments; lived amongst mantled howler *(Alouatta palliata)* and white-faced capuchin *(Cebus capucinus)* monkeys; and analyzed the effects of deforestation on these monkeys' genetic structures that I experienced a change of heart. I realized we are more connected to other animals and the environment that I had initially thought, and I suddenly became aware of how selfish and narrow-minded I had been.

This shift in focus from humans to animals and the environment is mirrored in the structure of my Honors Thesis. I began by establishing our human rights to our genetic information, and I stressed the importance of transparency and informed consent in genetic research involving humans (Chapter II). Humans are fortunate in that they can choose whether or not to participate in a study. Animals, on the other hand, cannot. In Chapter III, I discussed the ethics of using non-human primates, our closest relatives, in genetic research. I conceded that because non-human primates are sentient creatures, researchers must abide by the humane treatment principle and avoid inflicting unnecessary suffering on them. This chapter was inspired by my experiences in Costa Rica and the connections I developed with *A. palliata* and *C. capucinus*. I spent a month in the rainforest studying the social structure of *A. palliata* groups in relation to their distribution of food (Chapter IV). Studying the monkeys' behavior and living amongst them forced me to recognize how similar we were to them. Finally, I presented the results of my genetics study and illustrated how human-induced habitat fragmentation is negatively affecting the genetic structures of *A. palliata* and *C. capucinus* (Chapter V).

It is too often that we focus only on our human wants and needs without considering how our actions are affecting the lives of animals or the environment. We must recognize that we are not the most important organisms living on Earth. I see now that it is not ethical for us to consistently subject animals to pain and suffering on our behalf, and it is not morally permissible for us to destroy habitats without considering the consequences. Although I have acknowledged and embraced these problems, I have still struggled to draw the moral line between what is right or wrong, and I cannot offer any

final solutions to the animal rights or conservation issues. Indeed, I do not think we will ever be able to completely eradicate animal involvement in genetic studies or adopt a purely sustainable lifestyle; however, we are morally obligated to try. Therefore, I end on a quote by Annie Dillard (1999) from her book *For the Time Being,* in which she concedes, “The work is not yours to finish…but neither are you free to take no part in it” (p. 202). Recognizing that we are connected to other animals and to the environment is the first step in the right direction – the first step towards just coexistence with the earth and its non-human species.

References

American Type Culture Collection (ATCC). (2017). HeLa (ATCC® CCL-2™). Retrieved from https://www.atcc.org/products/all/CCL-2.aspx

Andorno, R. (2004). The right not to know: An autonomy based approach. *Journal of Medical Ethics, 30,* 435-440. doi:10.1136/jme.2002.001578

Altmann, J. (1974). Observational study of behavior: Sampling methods. *Behaviour, 49,* 227-267. doi:10.1163/156853974X00534

Andrews, C. (2010). The Hardy-Weinberg principle. *Nature Education Knowledge, 3,* 65. Retrieved from http://www.nature.com/scitable/knowledge/library/the-hardy-weinberg-principle-13235724

Arroyo-Rodriguez, V., & Dias, P. A. (2010). Effects of habitat fragmentation and disturbance on howler monkeys: a review. *American Journal of Primatology, 72,* 1-16. doi:10.1002/ajp.20753

Arroyo-Rodríguez, V., & Mandujano, S. (2006). Forest fragmentation modifies habitat quality for *Alouatta palliata*. *International Journal of Primatology, 27,* 1079-1096. doi:10.1007/s10764-006-9061-0

Barton, M. (2016). *Vegetation structure of a Costa Rican rainforest fragment and the genetic structure of the inhabitant Alouatta palliata population* (Undergraduate honors thesis). Retrieved from http://epublications.regis.edu/cgi/viewcontent.cgi?article=1702&context=theses

Bezanson, M., Garber, P. A., Murphy, J. T., & Premo, L. S. (2008). Patterns of subgrouping and spatial affiliation in a community of mantled howling monkeys *(Alouatta palliata)*. *American Journal of Primatology, 70,* 282-293. doi:10.1002/ajp.20486.

Chapman, C. (1987). Flexibility in diets of three species of Costa Rican primates. *Folia primatology, 49,* 90-105. doi:10.1159/000156311

Chen, Y., Zheng, Y., Kang, Y., Yang, W., Niu, Y., Guo, X., & ... Li, X. (2015). Functional disruption of the dystrophin gene in rhesus monkey using CRISPR/Cas9. *Human Molecular Genetics*, *24*, 3764-3774. doi:10.1093/hmg/ddv120

Chuecco, F. (n.d.) *The five great primates.* Retrieved from http://www.projetogap.org.br/en/primate/the-five-great-primates2/

Clarke, M. R., Collins, D. A., & Zucker, E. L. (2002). Responses to deforestation in a group of mantled howlers *(Alouatta palliata)* in Costa Rica. *International Journal of Primatology*, *23*, 365-381. doi:10.1023/A:1013839713223

Clarke, M. R., Zucker, E. L., & Scott Jr., N. J. (1986). Population trends of the mantled howler groups of La Pacifica, Guanacaste, Costa Rica. *American Journal of Primatology*, *11,* 79-88. doi:10.1002/ajp.1350110108

Cohen, C. (2001). In defense of the use of animals. In C. Cohen & T. Regan (Aut.), *The Animal Rights Debate* (pp. 3-40). Lanham: Rowman & Littlefield Publishers, Inc.

Cortés-Ortiz, L., Mondragón, E., Cabotage, J. (2009). Isolation and characterization of microsatellite loci for the study of Mexican howler monkeys, their natural

hybrids, and other Neotropical primates. *Conservation Genetics Resources, 2,* 21-26. doi:10.1007/s12686-009-9124-6

Crockett, C. M. & Eisenberg, J. F. (1987). Howlers: Variations in group size and demography. In B. Smuts, R.M. Cheyney, W. Seyfarth, R.M. Wrangham, & T.T. Struhsaker, *Primate Societies* (pp.54-62). Chicago: University of Chicago Press.

Crofoot, M. (2007). Mating and feeding competition in white-faced capuchins *(Cebus capucinus)*: The importance of short- and long-term strategies. *Behaviour, 144,* 1473-1495. doi:10.1163/156853907782512119

Dai, Y. Lin, Q. Fang, W. Zhou, X. & Chen, X. (2015). Noninvasive and nondestructive sampling for avian microsatellite genotyping: a case study on the vulnerable Chinese Egret *(Egretta eulophotes). Avian Research, 6,* 1-9. doi:10.1186/s40657-015-0034-x

Dillard, A. (1999). *For the Time Being*. New York: Vintage Books.

DNA Sequencing Core. (2016). *Interpretation of sequencing chromatograms*. Retrieved from https://seqcore.brcf.med.umich.edu/doc/dnaseq/interpret.html

Dolan DNA Learning Center. (2006). *Using an Alu insertion polymorphism to study human populations.* Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Fedigan, L. (1993). Sex differences and intersexual relations in adult white-faced capuchins *(Cebus capucinus). International Journal of Primatology, 14,* 853-877. doi:10.1007/BF02220256

Ferdowsian, H. R., Durham, D. L., Kimwele, C., Kranendonk, G., Otali, E., Akugizibwe, T., & ... Johnson, C. M. (2011). Signs of mood and anxiety disorders in chimpanzees. *Plos ONE, 6*, doi:10.1371/journal.pone.0019855

Francione, G. L. (2004). Animals – property or persons? In C. R. Sunstein & M.C. Nussbaum (Eds.), *Animal rights: Current Debates and New Directions* (pp. 108-142). New York: Oxford University Press.

Gallup, G. G. (1970). Chimpanzees: Self-recognition. *Science, 167*, 86-87. doi:10.1126/science.167.3914.86

Gallup, G. G., Wallnau, L. B., Suarez, S. D. (1980). Failure to find self-recognition in mother-infant and infant-infant rhesus monkey pairs. *Folia Primatologica, 33,* 210-219. doi:10.1159/000155935

Garber, P., Molina, A., & Molina, R. (2010). Putting the community back in community ecology and education: the role of field schools and private reserves in the ethical training of primatologists. *American Journal of Primatology, 72,* 785-793. doi:10.1002/ajp.20837

Garber, P. A., & Rehg J. A. (1999). The ecological role of the prehensile tail in white-faced capuchins (*Cebus capucinus*). *American Journal of Physical Anthropology, 110*, 325-339. doi:10.1002/(SICI)1096-8644(199911)110:3<325::AID-AJPA5>3.0.CO;2-D

Gebo, D.L. (1992). Locomotor and postural behavior in *Alouatta palliata* and *Cebus capucinus*. *American Journal of Primatology*, *26,* 277-290. doi:10.1002/ajp.1350260405

Genetics Home Reference. (2017). What is genetic discrimination? In *Help me understand genetics: Genetic testing.* Retrieved from https://ghr.nlm.nih.gov/primer/testing/discrimination

Glander, K. E. (1992). Dispersal patterns in Costa Rican mantled howling monkeys. *International Journal of Primatology, 13*, 415-435. doi:10.1007/BF02547826

Hallowell, N., Hall, A., Alberg, C., & Zimmern, R. (2015). Revealing the results of whole-genome sequencing and whole-exome sequencing in research and clinical investigations: some ethical issues. *Jounral of Medical Ethics, 41,* 317-321. doi:10.1136/medethics-2013-101996

Hamilton, J. G., Lobel, M., & Moyer, A. (2009). Emotional distress following genetic testing for hereditary breast and ovarian cancer: A meta-analytic review. *Health Psychology*, *28*, 510-518. doi:10.1037/a0014778

Harding, J. D. (2013). Progress in genetics and genomics of nonhuman primates. Introduction. *ILAR Journal*, *54*, 77-81. doi:10.1093/ilar/ilt051

Herring, J., & Foster, C. (2012). "Please don't tell me": The right not to know. *Cambridge Quarterly of Healthcare Ethics*, *21*, 20-29. doi:10.1017/S0963180111000466

Hiramatsu, C., Tsutsui, T., Matsumoto, Y., Aureli, F., Fedigan, L. M., & Kawamura, S. (2005). Color vision polymorphism in wild capuchins (*Cebus capucinus*) and spider monkeys (*Ateles geoffroyi*) in Costa Rica. *American Journal of Primatology*, *67*, 447-461. doi:10.1002/ajp.20199

International Genome Sample Resource. (2015a). *1000 Genomes Project: Developing a research resource for studies of human genetic variation.* Retrieved from http://www.internationalgenome.org/sites/1000genomes.org/files/docs/Informed%20Consent%20Form%20Template.pdf

International Genome Sample Resource (2015b). *Overview*. Retrieved from http://www.internationalgenome.org/sample_collection_principles

Islam, D., Ruamsap, N., Khantapura, P., Aksomboon, A., Srijan, A., Wongstitwilairoong, B., & ... Mason, C. J. (2014). Evaluation of an intragastric challenge model for Shigella dysenteriae 1 in rhesus monkeys *(Macaca mulatta)* for the pre-clinical assessment of Shigella vaccine formulations. *Apmis*, *122*, 463-475. doi:10.1111/apm.12168

Jack, K. M., & Fedigan, L. M. (2009). Female dispersal in a female-philopatric species, *Cebus capucinus*. *Behaviour*, *146*, 471-497. doi:10.1163/156853909X404420

Jinek, M., Alexandra, E., Cheng, A., Lin, S., Ma, E., & Doudna, J. (2013). RNA-programmed genome editing in human cells. *eLife,* 1-9. doi:10.7554/eLife.00471

Joh, E. E. (2011). DNA theft: Recognizing the crime of nonconsensual genetic collection and testing. *Boston University Law Review*, *91*, 665-700.

Jones, C. B. (1980). The functions of status in the mantled howler monkey, *Alouatta palliata* gray: intraspecific competition for group membership in a folivorous neotropical primate. *Primates, 21,* 389-405. doi:10.1007/BF02390468

Jones, W. T. & Bush, B. B. (1988). Darting and marking techniques for an arboreal forest monkey, *Cercopithecus ascanius*. *American Journal of Primatology, 14,* 83-89. doi:10.1002/ajp.1350140108

Juth, N. (2014). The right not to know and the duty to tell: The case of relatives. *Journal of Law, Medicine, & Ethics, 42,* 38-52. doi:10.1111/jlme.12117

Koenig, A. (2002). Competition for resources and its behavioral consequences among female primates. *International Journal of Primatology, 23*, 759-783. doi:10.1023/A:1015524931226

Laurie, G. T. (1999). In defence of ignorance: genetic information and the right not to know. *European Journal of Health Law, 6*(2), 119-132. doi:10.1163/15718099920522730

Markow, T., Hedrick, P. W., Zuerlein, K, Danilovs, J., Martin, J., Vyvial, T. & Armstrong, C. (1993). HLA polymorphism in the Havasupai: Evidence for balancing selection. *American Journal of Human Genetics, 53,* 943-952.

McDonald, P. G. & Griffith, S. C. (2011). To pluck or not to pluck: the hidden ethical and scientific costs of relying on feathers as a primary source of DNA. *Journal of Avian Biology, 42*, 197-203. doi:10.1111/j.1600.048X.2011.05365.x

McGuire, A. L. & Beskow, L. M. (2010). Informed consent in genomics and genetic research. *Annual Review of Genomics and Human Genetics, 11,* 361-381. doi:10.1146/annurev-genom-082509-141711

Mighell, A. J., Markham, A. F., & Robinson, P. A. (1997). Alu sequences. *FEBS Letters, 417,* 1-5. doi:10.1016/S0014-5793(97)01259-3

Milton, K. (1981). Food choice and digestive strategies of two sympatric primate species. *The American Naturalist, 117*, 496-505. doi:10.1086/283730

Muniz, L., Perry, S., Manson, J. H., Gilkenson, H., Gros-Louis, J., & Vigilant, L. (2006). Father-daughter inbreeding avoidance in a wild primate population. *Current Biology, 16*, R156-R157. doi:10.1016/j.cub.2006.02.055

Muniz, L., Perry, S., Manson, J. H., Gilkenson, H., Gros-Louis, J., & Vigilant, L. (2010). Male dominance and reproductive success in wild white-faced Capuchins (*Cebus capucinus*) at Lomas Barbudal, Costa Rica. *American Journal of Primatology, 72*, 1118-1130. doi:10.1002/ajp.20876

Muniz, L. & Vigilant, L. (2008). Isolation and characterization of microsatellite markers in the white-faced capuchin monkey (*Cebus capucinus*) and cross-species amplification in other New World monkeys. *Molecular Ecology, 8*, 402–405. doi:10.1111/j.1471-8286.2007.01971.x.

National Congress of American Indians. (n.d.). *Havasupai Tribe and the lawsuit settlement aftermath.* Retrieved from http://genetics.ncai.org/case-study/havasupai-Tribe.cfm

National Human Genome Institute. (2011). *Whole genome association studies*. Retrieved from https://www.genome.gov/17516714/

National Institutes of Health (NIH). (2007). *Analysis of rhesus monkey genome uncovers genetic differences with humans, chimps.* Retrieved from https://www.nih.gov/news-events/news-releases/analysis-rhesus-monkey-genome-uncovers-genetic-differences-humans-chimps

National Institutes of Health (NIH). (2011). *NIH-funded scientists publish orangutan genome sequence*. Retrieved from https://www.nih.gov/news-events/news-releases/nih-funded-scientists-publish-orangutan-genome-sequence

National Institutes of Health (NIH). (2016a). *Genetic discrimination.* Retrieved from https://www.genome.gov/10002077/

National Institutes of Health (NIH). (2016b). *NIH plan to retire all NIH-owned and -supported chimpanzees*. Retrieved from https://dpcpsi.nih.gov/orip/cm/chimpanzeeretirement

National Institutes of Health (NIH). (2016c). *An overview of the Human Genome Project.* Retrieved from https://www.genome.gov/12011238/an-overview-of-the-human-genome-project/

National Partnership for Women & Families. (2004). Faces of genetic discrimination: How genetic discrimination affects real people. Retrieved from http://go.nationalpartnership.org/site/DocServer/FacesofGeneticDiscrimination.pdf?docID=971

Nechvatal, J. M., Ram, J. L., Basson M. D., Nampracham, P., Niec, S. R., Badsha, K. Z., Matherly, L. H., Majumdar, A. P., & Kato, I. (2008). Fecal collection, ambient preservation, and DNA extraction for PCR amplification of bacterial and human markers from human feces. *Journal of Microbiological Methods, 72*, 124–132. doi:10.1016/j.mimet.2007.11.007

Neville, M. K., Glander, K. E., Brata, F., Rylands, A. B. (1988). The howling monkeys, Genus, *Alouatta*. In *Ecology and behavior of neotropical primates* (6). Retrieved from http://hdl.handle.net/10161/7086

Oklander, L. I., Kowalewski, M. M., & Corach, D. (2010). Genetic consequences of habitat fragmentation in black-and-gold howler (*Alouatta caraya*) populations from Northern Argentina. *International Journal of Primatology, 31,* 813-832. doi:10.1007/s10764-010-9430-6

Pauli, J. N., Whiteman, J. P., Riley, M. D., & Middleton, A. D. (2010). Defining noninvasive approaches for sampling of vertebrates. *Conservation Biology, 24,* 349-352. doi:10.1111/j.1523-1739.2009.01298.x

Peakall, R. & Smouse, P. (2009). Introduction to population genetic analysis. Material presented at the graduate workshop Introduction to Genetic Analysis for Populations Studies, Canberra, Australia

Perry, S. (1996). Intergroup encounters in wild white-faced capuchins (*Cebus capucinus*). *International Journal of Primatology, 17*, 309-330. doi:10.1007/BF02736624

Pope, T. (1992). The influence of dispersal patterns and mating system on genetic differentiation within and between populations of the red howler monkey (*Alouatta seniculus)*. *Evolution, 46,* 1112-1128. doi:10.2307/2409760

Presidential Commission for the Study of Bioethical Issues (2012). Privacy and progress in whole genome sequencing. *Genetic Alliance*, 2-140. Retrieved from geneticalliance.org/sites/default/files/webinararchive/privacysummary121213.pdf

Project R&R. (2017). *International bans.* Retrieved from http://www.releasechimps.org/laws/international-bans

Pruetz, J. D., & Leasor, H. C. (2002). Densities of primate species in forest fragments at La Suerte Biological Field Station, Costa Rica. *Neotropical Primates, 10,* 4-9.

Rivas, E. (2005). Recent use of signs by chimpanzees *(Pan troglodytes)* in interactions with humans. *Journal of Comparative Psychology, 119,* 404-417. doi:10.1037/0735-7036.119.4.404

Rodrigues, M. A. (2002). Age and sex-based differences in social interactions and spacing in mantled howling monkeys: implications for juvenile social development. *Ohio State University,* 103-114.

Roma, P. G., Silberberg, A., Huntsberry, M. E., Christensen, C. J., Ruggiero, A. M., & Suomi, S. J. (2007). Mark tests for mirror self-recognition in capuchin monkeys *(Cebus apella)* trained to touch marks. *American Journal of Primatology, 69,* 989-1000. doi:10.1002/ajp.20404

Ryan, S. J., Philip T. S., Milton, K. & Getz, W. M. (2008). Intersexual conflict and group size in *Alouatta palliata*: A 23-year evaluation. *International Journal of Primatology, 29,* 405-420. doi:10.1007/s10764-007-9172-2

Sandoe, P. & Holtug, N. (1996). Ethical limits to domestication. *Journal of Agricultural and Environmental Ethics, 9,* 114-122. doi:10.1007/BF03055297

Saulsberry, K. & Terry, S. F. (2013). The need to build trust: A perspective on disparities in genetic testing. *Genetic Testing and Molecular Biomarkers, 17,* 647-648. doi:10.1089/gtmb.2013.1548

Singer, P. (1975). *Animal Liberation.* New York: Harper Collins Publishers.

Skene, L. (2005). Theft of DNA: Do we need a new criminal offence? In J. Gunning & S. Holm (Eds.), *Ethics, Law and Society* (Vol. 1) (pp. 85-94). Burlington, VT: Ashgate Publishing Company.

Skloot, R. (2010). *The Immortal Life of Henrietta Lacks*. New York: Crown.

Steneck, N. H. (1997). Role of the Institutional Animal Care and Use Committee in monitoring research. *Ethics & Behavior*, *7*, 173. doi:10.1207/s15327019eb0702_8

Storz, J. F. (1999). Genetic consequences of mammalian social structure. *Journal of Mammalogy*, *80*, 553-569. doi:http://dx.doi.org/10.2307/1383301

Suddendorf, T., & Butler, D. L. (2013). The nature of visual self-recognition. *Trends in Cognitive Sciences*, *17*, 121-127. doi:10.1016/j.tics.2013.01.004

Taberlet, P., Waits, L. P., & Luikart, G. (1999). Noninvasive genetic sampling: look before you leap. *Trends in Ecology & Evolution, 14*, 323-327. doi:http://dx.doi.org/10.1016/S0169-5347(99)01637-7

Takala, T. (2001). Genetic ignorance and reasonable paternalism. *Theoretical Medicine and Bioethics: Philosophy of Medical Research and Practice*, *22*, 485-491. doi:10.1023/A:1013019120277

Tupasela, A. (2011). From gift to waste: changing policies in biobanking practices. *Science and Public Policy, 38*, 510-520. doi:10.3152/030234211X12960315268056

van Belle, S., Estrada, A., Strier, K. B., & Di Fiore, A. (2012). Genetic structure and kinship patterns in a population of black howler monkeys, *Alouatta pigra*, at

Palenque National Park, Mexico. *American Journal of Primatology, 74,* 948–957. doi:10.1002/ajp.22047

van Leeuwen, E. J., Mulenga, I. C., Bodamer, M. D., & Cronin, K. A. (2016). Chimpanzees' responses to the dead body of a 9-year-old group member. *American Journal of Primatology*, *78,* 914-922. doi:10.1002/ajp.22560

van Schaik, C. P. (1989). The ecology of social relationships amongst female primates. In V. Standen & R. A. Foley (Eds.), *Comparative Sociology: The Behavior Ecology of Humans and Other Mammals* (pp.195-217). Oxford: Blackwell Scientific.

Wall, J. D. (2013). Great ape genomics. *Institute for Laboratory Animal Research Journal, 54,* 82-90. doi:10.1093/ilar/ilt048

Wang, E. & Milton, K. (2002). Intragroup social relationships of male *Alouatta palliata* on Barro Colorado Island, Republic of Panama. *International Journal of Primatology*, *24,* 1227-1243. doi:10.1023/B:IJOP.0000005989.29238.ce

Wasserman, M. D., Chapman, C. A., Milton, K. A., Goldberg, T. L., Ziegler, T. E. (2013). Physiological and behavioral effects of capture darting red colobus monkeys *(Procolobus rufomitratus)* with a comparison to chimpanzee *(Pan troglodytes)* predation. *International Journal of Primatology*, *34,* 1020-1031. doi:10.1007/s10764-013-9711-y

Watkins, W. S., Ricker, C. E., Jorde, L. B., Bamshad, M. J., Harpending, H. C., Rogers, A. R., & ... Batzer, M. A. (2001). Patterns of ancestral human diversity: An analysis of Alu-insertion and restriction-site

polymorphisms. *American Journal of Human Genetics, 68,* 738-752. doi:10.1086/318793

Wikberg, E. C., Jack, K. M., Fedigan, L. M., Campos, F. A., Yashima, A. S., Bergstrom, M. L., & ... Kawamura, S. (2017). Inbreeding avoidance and female mate choice shape reproductive skew in capuchin monkeys (*Cebus capucinus* imitator). *Molecular Ecology, 26,* 653-667. doi:10.1111/mec.13898

Wikberg, E. C., Jack, K. M., Campos, F. A., Fedigan, L. M., Sato, A., Bergstrom, M. L., & ... Kawamura, S. (2014). The effect of male parallel dispersal on the kin composition of groups in white-faced capuchins. *Animal Behaviour, 96,* 9-17. doi:10.1016/j.anbehav.2014.07.016

Winkler, L. A., Xichen Zhang, L. A., Ferrell, R., Wagner, R., Dahl, J., Peter, G., & Sohn, R. (2004). Geographic microsatellite variability in Central American howling monkeys. *International Journal of Primatology, 25,* 197-210. doi:10.1023/B:IJOP.0000014650.86577.b4.

Wrangham, R. W. (1980). An ecological model of female-bonded primate groups. *Behavior, 75,* 264-300. doi:10.1163/156853980X00447

Wright, S. (1951). The genetical structure of populations. *Annals of Eugenics, 15,* 323-354. doi 10.1111/j.1469-1809.1949.tb02451.x

Yang, S., Cheng, P., Banta, H., Piotrowska-Nitsche, K., Yang, J., Cheng, E. H., & ... Chan, A. S. (2008). Towards a transgenic model of Huntington's disease in a non-human primate. *Nature, 453,* 921-924. doi:10.1038/nature06975

Zheng-Bradley, X. & Flicek, P. (2016). Applications of the 1000 Genomes Project resources. *Briefings in Functional genomics, elw027,* 1-8. doi:10.1093/bfgp/elw027

Zucker, E. L. & Clarke, M. R. (1998). Agonistic and affiliative relationships of adult female howlers *(Alouatta palliata)* in Costa Rica over a 4-year period. *International Journal of Primatology, 19,* 433-449. doi:10.1023/A:1020356321396.