Colors of Primate Pelage: The Independent Evolution of Sexual Dichromatism in the Primate Order

by

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There is a large body of research describing the evolutionary importance of plumage coloration among avian species. However, similar datasets are lacking for mammalian pelage. Furthermore, very little research has examined the variations of nonhuman primate (NHP) pelage coloration and patterning. Primatologists have noted conspicuous differences in coloration and patterning among NHPs, including neo-natal coats and sexual dichromatism. Sexual dichromatism refers to the differences in pelage coloration between the sexes of a single species. Sexual dichromatism is rare, but found among some species of lemurs, New World monkeys, and lesser apes. To illuminate the genetic mechanism of NHP sexual dichromatism, I examined published amino acid sequences for the MC1R and OCA2 genes of nine NHP species across multiple genera. This dataset incorporated sexually dichromatic NHPs including white-cheeked gibbons (*Nomascus leucogenys*), lar gibbons (*Hylobates lar*), and black howler monkeys (*Alouatta caraya*). I also examined closely allied monochromatic NHPs including brown lemurs *(Eulemur fulvus*), long-tailed macaques (*Macaca fascicularis*), black snub-nosed monkeys (*Rhinopithecus bieti*), Mueller’s gibbon (*Hylobates muelleri*), mantled howler monkeys (*Alouatta palliata*), and chimpanzees (*Pan troglodytes*). Comparisons across these species suggest the MC1R gene does not play an important role in pelage coloration. In contrast, the OCA2 sequence of N. leucogenys differed, on average, ~16% from the three monochromatic species. Furthermore, the OCA2 sequences exhibit a low phylogenetic signal, suggesting that this gene may regulate dichromatic pelage. To expand these genetic datasets, I analyzed socioecological variables among these species and found that smaller home-range sizes and dispersal of both sexes may have played a role in the evolution of dichromatic pelage in NHPs.

Colors of Primate Pelage: The Independent Evolution of Sexual Dichromatism in the Primate Order

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CHAPTER 1: Introduction

There is a wide array of pelage (i.e. fur or hair) coloration and patterns among the nearly 300 nonhuman primate (NHPs) species. This diversity serves a variety of functions, ranging from camouflage to conspicuous sexual signaling. Since the 19th century, natural historians noted the camouflage and signaling value of mammalian color patterns (Bradley and Mundy, 2008). However, despite a plethora of research on the evolutionary significance of coloration and pelage in other vertebrates, most notably birds, our understanding of the evolution of mammalian color has progressed very little in past decades. Furthermore, research on the function of NHP pelage coloration is almost non-existent and requires formal testing.

There have been some recent important contributions to the study of primate pelage, for example, the most complete lemur genome sequence for the sexually dichromatic blue-eyed black lemur (*Eulemur flavifrons*) (Meyer et al, 2015). At present, scientists are sequencing more NHP genomes with the advent of new genetic instrumentation and methods. This work is shedding new light on our understanding of skin pigmentation and pelage color throughout the Order Primates. However, these projects and publications frequently do not include comparisons across primate genera. Such comparisons can illuminate the genetic mechanisms behind different pelage patterns and provide data, which support the selective forces of convergent evolution. There is also a dearth of data regarding the genetic mechanisms of rare developmental pelage traits, including neo-natal coats. Neo-natal coats are conspicuous pelage colorations exhibited by the dependent offspring of some NHP species. These coats contrast the coloration exhibited by independent juveniles and adults (Treves, 1997). Of particular interest to this study are the genetic and socioecological traits associated with sexual dichromatism, drastic differences in pelage coloration between males and females of the same species (Treves, 1997).

I compare the genes linked to pelage coloration among nine NHPs across seven genera by comparing each species' amino acid sequences as detailed on the Uniport genetic database. The NHP species included in this review are brown lemurs (*Eulemur fulvus fulvus*), white-cheeked gibbons (*Nomascus leucogenys*), lar gibbons (*Hylobates lar*), Mueller's gibbons (*Hylobates muelleri*), mantled howler monkeys (*Alouatta palliata*), black howler monkeys (*Alouatta caraya*), black snub-nosed monkeys (*Rhinopithecus bieti*), long-tailed macaques (*Macaca fascicularis*), and chimpanzees (*Pan troglodytes*). Moreover, in this review, the genes associated with those NHPs that exhibit sexual dichromatic pelage, (white-cheeked gibbons, lar gibbons, and black howler monkeys), are compared to those NHP species that do not (chimpanzees, brown lemurs, long-tailed macaques, black snub-nosed monkeys, Mueller's gibbons, and mantled howler monkeys; Figure 1). These comparisons focus on the amino acid sequences linked to two genes that are known to play a key role in melanin production (*MC1R* and *OCA2*) (Suzuki, 2013; Meyer et al, 2015). By convention, genes will be presented in italics and proteins in regular font. In addition, this thesis incorporates socioecological data with an examination of the relationships among social structures, dispersal patterns, and mating patterns of those NHPs that exhibit sexually dichromatic pelage. A primary goal of these comparisons is to identify genomic differences between sister species in the same genus, and potential similarities between the three dichromatic NHPs that are not closely related.

In addition, this study aims to identify any conserved genetic sequences, which in evolutionary biology, are identical or similar sequences that occur in DNA and/or amino acid sequences. If sexual dichromatism evolved independently among NHPs lacking close evolutionary relationships, it is likely the outcome of convergent evolution whereby there has been selection for similar phenotypes independently across multiple primate lineages (Stayton, 2015). Overall, this research will provide useful data to broaden our understanding of mammalian and primate pelage coloration and patterning via genetics, social structures, and the environment. Furthermore, these results may illuminate the origins and evolution of hominin and human variations in hair color and placement across time and space.

CHAPTER 2: Background

Figure 1. Photographs of three nonhuman primate species exhibiting sexual dichromatism (A) white-cheeked gibbons (*Nomascus leucogenys*): male is on the right and female on the left, (B) black howler monkeys (*Alouatta* caraya): male on the right and female on the left, and (C) lar gibbons (*Hylobates lar*): male on the right and female on the left.

Table 1. Primate species, common name, presence or absence of sexual dichromatism, and social system

Species Name	Common Name	Exhibits Sexual Dichromatism (yes/no)	Monogamous (yes/no)
Pan troglodytes	Chimpanzee	No	No
Hylobates lar	Lar Gibbon	Yes	Yes
Hylobates muelleri	Mueller's Gibbon	No	Yes
Nomascus leucogenys	White-cheeked Gibbon	Yes	Yes
Alouatta caraya	Black Howler Monkey	Yes	No
Alouatta palliata	Mantled Howler Monkey	No	No
Rhinopithecus bieti	Black Snub-nosed Monkey	No	No
Macaca fascicularis	Long-tailed Macaque	No	No
Eulemur fulvus	Brown Lemur	No	No

Sexually dichromatic nonhuman primates and socioecological variables

Nonhuman primates (NHPs) not only vary in pelage color, but they also vary in a variety of social characteristics, including social structures and mating systems. The genus *Alouatta* is commonly referred to as the howler monkeys and consists of nine species (DiFiore et al, 2010). Of the nine *Alouatta* species, only the black howler monkey exhibits sexual dichromatism. Adult males and females in this genus disperse from natal groups at sexual maturity, which is rare among NHPs. (Rudran, 1979; Calegaro-Marques and Bicca-Marques, 1996). *Alouatta* intragroup female competition frequently results in “intense hostility” to immigrants, and females commonly force maturing females to emigrate (DiFiore and Fleischer, 2005; DiFiore et al, 2009). Adult males of the genus *Alouatta* may also react with “intense hostility” toward immigrating males and furthermore, males are frequently aggressive towards males in other social groups. Female and male dominance relationships within *Alouatta* groups consist of a social hierarchy, in which members of the group may interact in an aggressive context resulting in a ranking system (DiFiore et al, 2010). Unlike most Old World monkeys, females develop weak affiliative bonds with one another, as do *Alouatta* males. DiFiore et al. (2010) also noted that females of *Alouatta* sometimes solicit copulations from males and copulate outside of the periovulatory period. Occasionally, females will mate with multiple males and subordinate males may form consortships with receptive females, preventing dominant males from exclusively mating with the females.

When looking specifically at the black howler monkey (*A. caraya*), the species exhibit both sexual dimorphism and sexual dichromatism. Adult males exhibit a black coat, and females exhibit a blonde coat. The natal coat color for both sexes is blonde, and a male's fur will gradually darken from blonde to black with maturity (Bicca-Marques and Calegaro-Marques, 1998).

Male black howlers can weigh up to 10.0 kg and females weigh approximately 6.5 kg, with a body size dimorphism of 1.48, the male to female body size ratio (Bicca Marquez and Calegaro Marquez 1998**;** Malinow and Maruffo, 1966). The mating system of *A. caraya* is polygynandrous. On the other hand, the closely related mantled howler monkeys (*A. palliata*) exhibits sexual dimorphism**,** but no sexual dichromatic pelage. Male mantled howlers weigh on average 6.5 kg and females average 4.5 kg, with a body size dimorphism of 1.34 (Reid 1997, Glander 1983). Howler monkeys live in cohesive social groups, and most *Alouatta* species' groups consist of 10-15 animals and generally one adult male per group, but rarely not more than three (DiFiore et al, 2010). However, mantled howlers have similar groups, wherein one can contain three or more adult males and up to nine or more adult females (Fedigan et al, 1985**;** Chapman, 1988). The genus *Alouatta* is characterized by overlapping home-ranges. However, there is variation in the mean home-range size, and the variation within *A. palliata* is often greater than between other species of howler monkeys (Crockett, 1987). For example, the home-range size for black howlers is 1.7 to 5.5 hectares while the range for mantled howlers is 9.9 to 108 hectares (Bravo and Sallenave, 2003**;** Zunino and Rumiz, 1986**;** Glander, 1978; Chapman, 1988). Interestingly, the mean home-range is drastically lower in black howlers, the sexually dichromatic species in comparison to the monochromatic mantled howler monkey.

The genus *Eulemur* includes seven lemur species and several subspecies wherein some species exhibit sexual dichromatic pelage and others do not (Gould et al, 2007). Most members of the genus are characterized by cathemeral activity pattern, that is neither predominately nocturnal nor diurnal (Overdorff and Rassmussen, 1995). The social organization for *Eulemur* species is mixed-sex groups or fission-fusion groups (Sussman, 1974). Among the eight *Eulemur* species, the blue-eyed black lemur (*Eulemur flavifrons*) and the black lemur (*E. macaco*) both exhibit sexually dichromatic fur. The blue-eyed black lemur is also one of the least-studied lemurs and is listed as critically endangered (Meyer et al, 2015). Blue-eyed black lemurs are arboreal, frugivorous, and their social organization is multimale/multifemale, with some fissioning observed in larger groups (Colquhoun, 1993; Volampeno et al, 2011). Sexual dichromatism is marked in this species with the males exhibiting black fur, and the females exhibit reddish-orange fur color (Volampeno et al, 2011). There is little sexual dimorphism among blue-eyed black lemurs and on average, they weigh approximately 1.8 kg (Mittermeier, 1994). Unfortunately, the genetic data for these species present incomplete. At present, less than 80% of the genome have been published and there is no data on the amino acid sequences for either the MC1R or OCA2 proteins for this species (Meyer et al, 2015). Therefore, to include the *Eulemur* genus in the comparison of genera, this project will analyze the amino acid sequences of a monochromatic species, the brown lemur (*Eulemur fulvus fulvus*), and compare their social structures, mating patterns, and life-history traits to that of the blue-eyed black lemurs and the black lemurs, as well as the other NHP species of focus. Black lemurs, and brown lemurs live in dry forests, with brown lemurs living predominately in western portions of Madagascar, and black lemurs are found in northwestern portions of the island (Gould et al, 2007). As observed in the black lemurs, there is also little sexual dimorphism exhibited by brown lemurs, wherein the average weight is 2.6 kg. The mean group size of brown lemurs is 12 individuals and have a mean home-range size of about 7-20 hectares, in contrast the black lemurs average group size is 10 individuals with a home-range of 3.5-7 hectares (Harrington, 1975**;** Ganzhorn, 1988**;** Mittermeier, 1994**;** Colquhoun, 1993**;** Andrews and Birkinshaw, 1998). These data suggest that home-range size may act as factor that selected for dichromatic pelage potentially via an increase in mate competition. Both black and brown lemurs have similar mean group sizes; however, *E. macaco* is confined to smaller geographical ranges, resulting in more competition for contested food resources and mates. This pattern is similar to what has been observed among *Alouatta* species, whereby the dichromatic species (*A. caraya*) exhibits a smaller mean home-range size than that of the monochromatic species (*A. palliata*).

The remaining two sexual dichromatic species are both gibbons from two different genera, *Hylobates* and *Nomascus.* For the sake of comparison, the monochromatic Mueller's gibbon (*H. muelleri)* will also be included. Gibbons are considered lesser apes, and they exhibit several ancestral characteristics that are absent in most great apes (Bartlett, 2007). Gibbons possess ischial tuberosities, callus-like sitting pads on the lower portion of the pelvis found among Old World monkeys. Gibbons also exhibit highly derived morphological traits related to brachiation, including extremely long arms, as well as elongated and curved fingers. Gibbons also use loud calls, or songs, that are structurally complex. The current hypothesis is that these songs serve both to increase pair bonding and to advertise gibbon territory (Geissmann, 2002). Gibbons are considered socially monogamous primates. However, recent observations have revealed that lar gibbons (*H. lar*) and siamangs (*Symphalangus syndactlyus*), invoke an alternative sexual strategy that results in mating with already paired neighbors during intergroup encounters (Bartlett, 2007). Like the Great Apes, gibbons have longer and more complex life-history traits in comparison to other haplorrhines and strepsirrhines (Reichard and Barelli, 2008). In general, gibbons travel in small social groups, consisting of a bonded adult pair, and one to three offspring. Male and female gibbons disperse from their natal groups, a pattern also observed among black howlers and blue-eyed black lemurs. However, Brockelman et al. (1998), found no evidence of sub-adults being forced out of their natal group via intrasexual aggression. Female gibbons usually give birth to one offspring after a gestation period of about seven months, with an inter-birth-interval of two to four years (Napier and Napier, 1994; Geissmann, 1991). Furthermore, gibbons are unique amongst the apes in that males and females are sexually monomorphic. As such, males and females exhibit the same body size, and both sexes possess large canines with sectorial lower premolars for honing the upper canines. (Groves, 1972, 1984). Lar gibbons weigh 4.4 to 7.6 kg (Gron, 2010). Unlike other dichromatic NHPs, lar gibbons exhibit a variety of pelage colors; ranging from black, buff (i.e. pale orange-brown color), and dark brown pelage. In contrast, white-cheeked gibbons (*N. leucogenys*) weigh 5.6 to 5.8 kg and males exhibit black pelage while females exhibit buff pelage. Mueller's gibbons weigh 5.0 to 6.4 kg and both sexes exhibit brown to gray pelage (Bartlett, 2007). Most gibbon species live in allopatric home-ranges that are isolated and do not overlap geographically with other gibbon species. However, siamangs are sympatric with lar gibbons and agile gibbons (*H. agilis*) (Gittins and Raemaekers, 1980). The home-range size for lar gibbons is ~40 hectares and is 30.3 to 40.5 hectares for white-cheeked gibbons. The home-range for Mueller's gibbons is on average 44 hectares (Bartlett, 2007).

Sexually monochromatic nonhuman primates and socioecological variables

Although the primary goal of this project is to compare dichromatic NHP species across genera within the Order Primates, for comparative purposes some species for which genetic data are available are also included in this review that exhibit monochromatic pelage. The long-tailed macaque (*Macaca fascicularis*) is included to represent an Old-World monkey species. The dependent offspring of long-tailed macaques exhibit neo-natal coats. Among long-tailed macaques, infants exhibit dark black pelage and as they age into older infants and juveniles they become a mixture of gray and brown pelage (Aldrich-Blake, 1980). Long-tailed macaques are sexually dimorphic, and males have an average weight of 5.4 kg and females' average weight is 3.6 kg. They live in multi male and multi female groups and have a promiscuous mating system (Smith and Jungers, 1997**;** Dittus 2004). The mean home-range size of free-ranging long-tailed macaques is ~125 hectares, which is more than double the size of the home-ranges of the three sexually dichromatic species previously discussed (Wolfheim, 1983).

In addition, the black snub-nosed monkey (*Rhinopithecus bieti*) exhibits neonatal coloration but is not as drastic as the long-tailed macaques; instead, these infant monkeys exhibit a dark flat cap and a white or pale gray coat with a yellow or red tint on their bodies **(**Poirier and Hongxhin, 1983**).** Black snub-nosed monkeys are Asian colobines that primarily consume leaves. This species is found in southwest China's conifer forests and live in groups that usually consist of no more than 15 individuals. Most groups consist of only one adult male and these "one-male groups" travel together as a band (Kirkpatrick et al, 1998). Black snub-nosed monkeys are large semi-terrestrial primates which exhibit sexual dimorphism. The males weigh approximately 15 kg and possess large canines and in contrast females weigh about 8 or 9kg. (Kirkpatrick and Grueter, 2010). Groups can have extremely large home-range sizes, ranging from 1600 to 4000 hectares, this large variation is partly due to group sizes (Meyer et al, 2017). So, although group size may act as an important factor in determining home-range size, it is possible that the large home-ranges for black snub-nosed monkeys created an environment that did not positively select dichromatic pelage.

Chimpanzees (*Pan troglodytes*) are the closest living relative to *Homo sapiens*. Furthermore, including chimpanzees into this comparison could provide insight on the development of pelage pattern in nonhuman primates and create a parallel comparison useful to understanding hair location and color in both extinct and extant hominin species. Chimpanzees exhibit sexual dimorphism, with males being on average 5% to 14% heavier than females (Smith and Jungers, 1997). On average, male chimps weigh 42.7-59.7 kg and females range from 33.7 to 45.8 kg (Smith and Jungers, 1997). Chimpanzees do not exhibit sexually dichromatic pelage, however across subspecies there is variation amongst the color of faces. Furthermore, chimpanzees are born with a white tail tuft, that is not exhibited in adult chimpanzees (Stumpf, 2011). Chimpanzees live in fission-fusion social systems, in which individuals from large communities, occasionally form smaller subgroups, which can change in size and composition throughout the day (Boesch and Lehmann, 2003). It is hypothesized that this system may reduce intragroup feeding competition, as well as improve the efficiency of foraging (Stumpf, 2011). Though their groups consist of multiple males and multiple females, the dominant sex is male and adolescent females disperse from their natal groups (Pusey, 1979). Chimpanzees utilize a great diversity of habitat types throughout equatorial Africa, ranging from Southern Senegal to Western Tanzania. Chimpanzees are found primarily in tropical rainforests but also inhabit forest-savanna mosaics and mountain forests environments (Goodall, 1986; Jones et al, 1996; Napier and Napier, 1994; Nowak and Walker, 1999). As a species that does not exhibit dichromatic pelage, they follow the recognized pattern of having a large home-range size, ranging from 1200 to 2300 hectares (Boesch and Lehmann, 2003). However, this could be a result to other factors including body size, group size, and resource dispersion. For example, savanna chimpanzees have very large home-ranges because much of their habitat does not yield plants that supply them with adequate nutrition forcing them to move over long distances to forage for food (Pruetz and Bertolani, 2009).

Genomics and pelage coloration in nonhuman primates

In mammals, extensive research on the genomics of pigmentation has led to the discovery of about 100 genes related to pelage color (Barsh, 1996; Suzuki, 2013). The majority of research discussed in this review focuses primarily on two genes, the *MC1R* (Melanocortin-1 receptor) and the *OCA2* (melanocyte-specific transporter) (Suzuki, 2013). Melanocytes are the only cells that produces pigments in mammals and are regulated by the MC1R and the ASIP proteins (Agouti Signaling Protein; Table 2). Both proteins are essential for pelage color expression via the production of pheomelanin (red-yellow melanization) and eumelanin (black-brown melanization). The MC1R is a G-protein-coupled receptor that is expressed primarily in melanocytes in response to two types of signaling molecules, the α-melanocyte stimulating hormone (α-MSH) and ASIP (Suzuki, 2013). α-MSH functions as an agonist to the MC1R complex, which activates melanocytes via an increase in cAMP (Cyclic adenosine monophosphate, acts as a second messenger in signal transduction) levels. This cell activation starts the production of eumelanin. In contrast, the inverse agonist of the MC1R system, ASIP, which is released from dermal papilla cells at the base of hair follicles, deactivates melanocytes, resulting in the default production of pheomelanin (Suzuki, 2013). The MC1R system has seven transmembrane domains and an amino acid length of 317, with some exceptions. The *MC1R* protein is a single exon gene and mutations can change the affinity of the receptor, which could result in a possible new phenotypic expression of pelage color. *ASIP* consists of the peptides of 131 amino acids with a "cysteine-rich" region near the C-terminus (the last end of polypeptide chain) which is conserved across taxa (Suzuki, 2013). Pelage color is determined by the density of melanin as well as the distribution of melanin types on individual hair follicles. Allelic variation, and mutations that occur may lead to a wide degree of variation in pelage color within mammals. Allelic variation with reference to this pigmentation system can be classified as loss-of-function or gain-of-function. A loss-of-function results in solid hair patterns of red-yellow coloration and gain-of-function results in solid hair patterns of black-brown coloration. Extreme yellow pelage color is the consequence of destructive MC1R mutations (Suzuki, 2013). In addition, there are other proteins that play important roles in pelage color, such as OCA2 or TYR, which influence white and yellow colorings (Suzuki, 2013.

Since sexual dichromatic pelage is found in very few NHP species across genera, it is likely the result of convergent evolution. Convergent evolution provides an explanatory framework to understand patterns of sexual dichromatism and can help determine similarities and differences in genetic mechanisms. For this reason, comparing genomes of these three dichromatic species' genomes is important. Genome assembles of single diploid individuals provide the opportunity to estimate the population genetic parameter, or the nucleotide diversity. This is important because it shows the variation within a population and/or species. For example, examining two lemur species that both exhibit dichromatic pelage, blue-eyed black lemurs (*E. flavifrons*) and black lemurs (*E. macaco*), the genome wide diversity is 0.174% and 0.219%, respectively. Furthermore, the genome wide diversity of the blue-eyed black lemurs is lesser than most gibbon species, which only stresses the importance of analyzing and understanding their genome (Meyer et al, 2015). Meyer et al (2015) compared the genome wide diversity between the two black lemur species, and they identified candidate regions for allelic variation by searching for large regions with low within-species diversity relative to the total number of polymorphic sites (Meyer et al, 2015). Their analysis identified a region containing the orthologs of human genes *OCA2* and *HERC2* among regions with the most extreme signatures of selection in blue-eyed black lemurs. To evaluate the potential signal of directional selection in that region, they sequenced more individuals of each species. In the larger data set, the strongest signal was within the region that overlap the *OCA2* gene (Meyer et al, 2015). They also investigated patterns of genetic variation of sixteen other genes that have been known to be associated with iris pigmentation in humans. After correcting their data with resequencing and using gene ontology (GO) they found multiple genes with unusual differentiation including, *ASIP*, *OCA2*, *MITF*, *KITLG*, *TYR*, *FIG4*, *TCF7*, and *SERPINB2* genes (Meyers et al, 2015). Some of these genes were found to have vary between the closely related black and blue-eyed lemurs (*ASIP*, *OCA2*, *MITF*, *KITLG*, *TYR*, *FIG4*, *TCF7*). However, some of these genes do not have a direct link to pigmentation nor melanin production and/or the amino acid sequences have not been sequenced for some of the NHP species investigated here. Therefore, this review focuses on the previous described *MC1R* gene, and the *OCA2* gene which appears to be conserved amongst the two species of lemurs (Meyer et al, 2015). The OCA2 melanosomal transmembrane protein, provides the instructions for making the P protein, which is located within melanocytes. The exact function of the P protein is unknown, but it is understood that it is essential for normal pigmentation, therefore it is possible that mutations in this protein could play an important role in creating the variation of pelage color (NCBI Gene Data Base).

Table 2. Primate genes associated with pigmentation and their location in humans

Gene	Gene locus in *Homo sapiens*	Function
MC1R	Chromosome 16	Activates melanocytes via an increase in cAMP levels. This cell activation starts the production of eumelanin
ASIP	Chromosome 20	Helps to deactivate melanocytes, which leads to the default production of pheomelanin
OCA2	Chromosome 15	Provides the instructions for making the P protein and essential for normal pigmentation

Nonhuman primate phylogeny and sexual dichromatism

Comparing NHP species that either exhibit sexual dichromatic or monochromatic pelage can shed light on how phylogenetic divergence may have played a role in the evolution of sexual dichromatic pelage. The phylogenetic relationships of the existing 12 gibbon species have been inferred from short or partial mitochondrial DNA data (Matsudaira and Ishida, 2010). Therefore, Matsudaira and Ishida (2010) sequenced whole mitochondrial genome of *Hylobates agilis*, *H. pileatus*, *Nomascus* sp., and *Symphalangus syndactylus.* They used the Bayesian statistical models to estimate the divergence of these species and found that it is most likely that the species within the genus *Hylobates* diverged from the other gibbons in the Pliocene (Matsudaira and Ishida, 2010). They also hypothesize the *Nomascus* genus diverged from the other gibbon genera about 8.0 million years ago, the *Symphalangus* genus diverged from *Hylobates* genus about 7.0 million years ago. *H. pileatus* appears to have diverged from the other species in the genus *Hylobates* around 3.9 million years ago (Matsudaira and Ishida, 2010). Their estimated divergence times among the gibbon species is significant as they suggest that the *Nomascus* genus split from other gibbon genera first, followed by the dichromatic pileated gibbon. These findings support the hypothesis that sexual dichromatic fur evolved independently.

The total number of NHP species varies among primatologists, depending on inclusions of subspecies. None the less, most estimates suggest there are 300 NHP (Groves and Napier 2019). The NHP species that are considered sexually dichromatic (less than 1%) are greatly outweighed by the number of NHP species that exhibit monochromatic pelage. Given that sexually dichromatic pelage is a rare trait and found in genera that are not closely related, it appears to be the product of convergent evolution and comparing the amino acid sequences of the specific proteins (MC1R and OCA2) of sexually dichromatic and monochromatic NHPs can shed a light on the genetic mechanism. Furthermore, whether the genetic mechanism is similar or not, dichromatic pelage has been positively selected for lar gibbons, white-cheeked gibbons, black howler monkeys, blue-eyed black lemurs, and pileated gibbons. To determine if these socioecological variables are favorably selecting for dichromatism, one would be required to sequence the complete genomes of sexually dichromatic NHPs and their nearest relatives. Such a comparison would provide more useful information regarding the regions of DNA that have a direct relationship with pelage color and pigmentation in the Order primates.

CHAPTER 3: Hypotheses

This project assumes that sexual dichromatism is a product of convergent evolution because it is more likely that dichromatic pelage evolved independently in a few NHP species rather than the majority of NHP species which lost this trait over time. Dichromatic pelage may have some selective advantages, including increasing the likelihood of mate selection or camouflage of infants since in most dichromatic species, infants exhibit the female pelage pattern. Furthermore, as some studies in bird plumage suggest, pelage coloration in dichromatic NHPs could be an indicator of parasite resistance and immune capacity, although bird coloration is frequently linked to carotenoids, not melanin (Hamilton and Zuk, 1982).

> **H_1** - It is expected that there will be differences between the amino acid sequences of the three sexually dichromatic species; however, it is also likely that there will be conserved regions between the dichromatic species.
>
> **H_2** - I expect that the *MC1R* and *OCA2* genes will play a significant role in the genetic mechanism of sexually dichromatic pelage since their functions directly influence the production and preservation of melanin and melanocytes. The *OCA2* gene also provides instructions for making the P protein which is important in regulating pigmentation.
>
> **H_3**- Since socioecological variables may also have played a key role in the evolution of dichromatic pelage, I expect that the exhibition of dichromatic pelage may be linked to smaller group sizes and dispersal patterns.

CHAPTER 4: Methods

The molecular data used for this study were the amino acid sequences for the MC1R and OCA2 proteins. Nine nonhuman primate (NHP) species were included to perform comparisons of the amino acid sequences across seven genera. The list of amino acids and there corresponding one-letter abbreviation is included in Table 3. Only three of the nine species exhibit sexually dichromatic pelage (black howlers, lar gibbons, and white-cheek gibbons; see Table 1). The remaining six species are included to provide a more robust comparison (chimpanzees, Mueller's gibbons, black snub-nosed monkeys, long-tailed macaques, brown lemurs, and mantled howlers).

Table 3. Amino acids and their corresponding one letter abbreviations

1-Letter Abbreviation	Amino Acid
A	Alanine
R	Arginine
N	Asparagine
D	Aspartic acid
C	Cysteine
Q	Glutamine
E	Glutamic acid
G	Glycine
H	Histidine
I	Isoleucine
L	Leucine
K	Lysine
M	Methionine
F	Phenylalanine
P	Proline
O	Pyrrolysine
S	Serine
U	Selenocysteine
T	Threonine
W	Tryptophan
Y	Tyrosine
V	Valine
B	Aspartic acid or Asparagine
Z	Glutamic acid or Glutamine

The amino acid sequences analyzed for this study were available online from the UniProt library (https://www.uniprot.org/). UniProt is a free database of protein and amino acid sequences. This database is useful for comparing the relationships between organisms based on their protein sequences which are the product of genetic sequences.

In order to perform the comparisons between amino acid sequences, a computer program was created using JavaScript and HTML technology. The program identified differences between corresponding amino acids. This was performed by marking every occurrence where the amino acid in the first sequence differed from the amino acid in the same location in the second sequence. The differences were noted in the data output by both bolding and changing the font color of differing amino acids (red for the first sequence, blue for the second; see Figure 2). The computer program only allowed for comparisons between sequences that were of equal length, which was not problematic for the MC1R comparison, but some issues arose within the OCA2 comparison. When comparing the OCA2 sequences of the chimpanzee (*Pan troglodytes*), white-cheeked gibbon (*Nomascus leucogenys*), black snub-nosed monkey (*Rhinopithecus bieti*), and the long-tailed macaque (*Macaca fascicularis*), the length of the sequences were highly variable. The length of these sequences ranged from 775 to 838 amino acids. In order to use the previously described computer program, these sequences were edited to be an equal length, this process is described in more detail below.

Figure 2. An example of data output from the computer program used to generate amino acid sequence comparisons

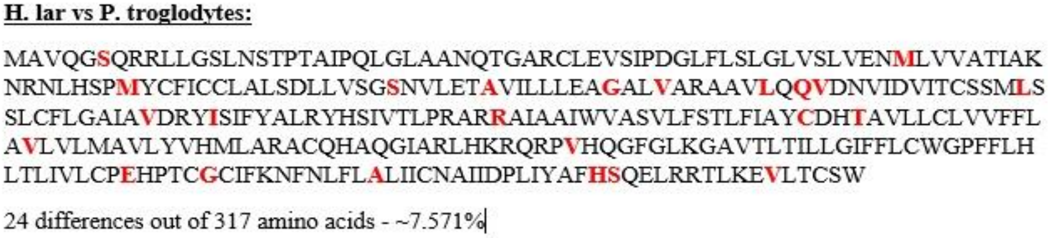
```
MAVQGFQRRLLGSLNSTPTAIPQLGLAANQTGARCLEVSIPDGLFLSLGLVSLVENVLVVATIAKNRNLHSPTYCFICCLALSDLLVSGGNVLETVVILLLEASALAARAAVVQPLDNVIDVITCSSMVSSLCFLGAIAMDRYVS
IFYALRYHSIVTLPRARQAIAAIWVASVLFSTLFIAYYDHAAVLLCLVVFFLAMLVLMAVLYVHMLARACQHAQGIARLHKRQRPLHQGFGLKGAVTLTILLGIFFLCWGPFFLHLTLIVLCPQHPTCSCIFKNFNLFLTLIICN
AIIDPLIYAFRRQELRRTLKEGLTCSW
```

```
MAVQGSQRRLLGSLNSTPTAIPQLGLAANQTGARCLEVSIPDGLFLSLGLVSLVENMLVVATIAKNRNLHSPMYCFICCLALSDLLVSGSNVLETAVILLLEAGALVARAAVLQQVDNVIDVITCSSMLSSLCFLGAIAVDRYIS
IFYALRYHSIVTLPRARRAIAAIWVASVLFSTLFIAYCDHTAVLLCLVVFFLAVLVLMAVLYVHMLARACQHAQGIARLHKRQRPVHQGFGLKGAVTLTILLGIFFLCWGPFFLHLTLIVLCPEHPTCGCIFKNFNLFLALIICN
AIIDPLIYAFHSQELRRTLKEVLTCSW
```

```
MAVQGFQRRLLGSLNSTPTAIPQLGLAANQTGARCLEVSIPDGLFLSLGLVSLVENVLVVATIAKNRNLHSPTYCFICCLALSDLLVSGGNVLETVVILLLEASAL
MAVQGSQRRLLGSLNSTPTAIPQLGLAANQTGARCLEVSIPDGLFLSLGLVSLVENMLVVATIAKNRNLHSPMYCFICCLALSDLLVSGSNVLETAVILLLEAGA
```

The MC1R sequence of each NHP contained 317 amino acids. Using the computer program described above, each NHP species had its MC1R sequence compared to the MC1R sequences of remaining species included in this analysis. For example, lar gibbons and chimpanzees had 24 differences of 317 total amino acids. This resulted in a 7.517% difference between the sequences of these two NHP species (Figure 3). The amino acid sequence for the OCA2 protein was only available for four of the nine NHP species used in the MC1R comparison (chimpanzees, black snub-nosed monkeys, white-cheeked gibbons, and long-tailed macaques). Furthermore, the number of amino acids in each OCA2 sequence varied with each species. For example, chimpanzees and black snub-nosed monkeys both have 838 amino acids, while white-cheeked gibbons and long-tailed macaques has 804 and 775, respectively. As noted previously, to develop an equal comparison between the species, the amino acid sequences were reduced to match the species with the shortest sequence, either the long-tailed macaque or white-cheeked gibbon. Shorter amino acid sequences were constructed by identifying and removing unique groups of amino acids from the species with the longer sequence. For the purpose of this process, a unique group is defined as any group of amino acids that does not have a homologous sequence in the other species. Homologous sequences are sequences that look similar to each other and appear to share common ancestry but may or may not display the same activity. An example of this sequence shortening process can be observed with the chimpanzee. Its sequence was reduced from 838 amino acids to 804 to allow for a suitable comparison with the white-cheeked gibbon (Figure 4).

Figure 3. MC1R sequence comparison between lar gibbons (*H. lar*) and chimpanzees (*P. troglodytes*)



H. lar vs P. troglodytes:

MAVQG**S**QRRLLGSLNSTPTAIPQLGLAANQTGARCLEVSIPDGLFLSLGLVSLVEN**M**LVVATIAK
NRNLHSP**M**YCFICCLALSDLLVSG**S**NVLET**A**VILLLEA**G**AL**V**ARAAV**L**Q**QV**DNVIDVITCSSM**L**S
SLCFLGAIA**V**DRY**I**SIFYALRYHSIVTLPRAR**R**AIAAIWVASVLFSTLFIAY**C**DH**T**AVLLCLVVFFL
A**V**LVLMAVLYVHMLARACQHAQGIARLHKRQRP**V**HQGFGLKGAVTLTILLGIFFLCWGPFFLH
LTLIVLCP**E**HPTC**G**CIFKNFNLFL**A**LIICNAIIDPLIYAF**HS**QELRRTLKE**V**LTCSW

24 differences out of 317 amino acids - ~7.571%

Figure 4. Length reduction in the OCA2 sequence in chimpanzees (*P. troglodytes*)

Pan troglodytes: [UniProtKB - A0A2I3TCV4]

MHLEGRDGRRYPGTPAVELLQTSVPTGLAELAAGKHRLPRGAGGADPSHSCPSGAAGQSSWAP
AGQEFASFLTKGRSHSSLPQMSSSRSKDSCFTENTPLLRNSLQEKGSRCIPVYHPEFITAEESWEDS
SADWERRYLLSREVSGLSASASSEKGDLLDSPHIRLRLSKLRRCVQWLKVTGLFVFVVLCSILFSL
YPDQGKLWQLLALSPLENYSVNLSSHVDSTLLQVDLAGALVASGPSRPGREEHIVVELTQADAL
GSRWRRPQQVTHNWTVYLNPRRSEHSVMSRTFEVLTRETVSISIRASLQQTQAVPLLMAHQYLR
GSVEAQVTIATAILAGVYALIIFEIVHRTLAAMLGSLAALAALAVIGDRPSLTHVVEWIDFETLAL
LFGMMILVAIFSETGFFDYCAVKAYRLSRGRVWAMIIMLCLIAAVLSAFLDNVTTMLLFTPVTIR
LCEVLNLDPRQVLIAEVIFTNIGGAATAIGDPPNVIIVSNQELRKMGLDFAGFTAHMFIGICLVLLV
CFPLLRLLYWNRKLYNKEPSEIVELKHEIHVWRLTAQRISPASREETAVRRLLLGKVLALEHLLA
QRLHTFHRQISQEDKNWETNIQELQKKHRISDGILLAKCLTVLGFAIFMFFLNSFVPGIHLDLGWI
AILGAIWLLILADIHDFEIILHRVEWATLLFFAALFVLMEALAHLHLIEYVGEQTALLIKMVPEEQ
RLTAAIVLVVWVSALASSLIDNIPFTATMIPVLLNLSHDPEVGLPAPPLMYALAFGACLGGNGTLI
GRDGRQ GASANVVCAGIAEQHGYGFSFMEFFRLGFPMMVVSCTVGMCYLLVAHVVVGWN
[838]

MHLEGRDGRRYPGTPAVELLQTSVPTGLAELAAGKHRLPRGAGGADPSHSCPSGAAGQSSWAP
AGQEFASFLTKGRSHSSLPQMSSSRSKDSCFTENTPLLRNSLQEKGSRCIPVYHPEFITAEESWEDS
SADWERRYLLSREVSGLSASASSEKGDLLDSPHIRLRLSKLRRCVQWLKVTGLFVFVVLCSILFSL
YPDQGKLWQLLALSPLENYSVNLSSHVDSTLLQVDLAGALVASGPSRPGREEHIVVELTQADAL
GSRWRRPQQVTHNWTVYLNPRRSEHSVMSRETVSISIRASLQQTQAVPLLMAHQYLRGSVEAQ
VTIATAILAGVYALIIFERPSLTHVVEWIDFETLALLFGMMILVAIFSETGFFDYCAVKAYRLSRGR
VWAMIIMLCLIAAVLSAFLDNVTTMLLFTPVTIRLCEVLPRQVLIAEVIFTNIGGAATAIGDPPNVII
VSNQELRKMGLDFAGFTAHMFIGICLVLLVCFPLLRLLYWNRKLYNKEPSEIVELKHEIHVWRLT
AQRISPASREETAVRRLLLGKVLALEHLLAQRLHTFHRQISQEDKNWETNIQELQKKHRISDGILL
AKCLTVLGFAIFMFFLNSFVPGIHLDLGWIAILGAIWLLILADIHDFEIILHRVEWATLLFFAALFVL
MEALAHLHLIEYVGEQTALLIKMVPEEQRLTAAIVLVVWVSALASSLIDNIPFTATMIPVLLNLSH
DPEVGLPAPPLMYALAFGACLGGNGTLIGASANVVCAGIAEQHGYGFSFMEFFRLGFPMMVVSC
TVGMCYLLVAHVVVGWN**[804]**

I also compared and analyzed socioecological variables of the nine NHP species examined in this study from data published from field studies. These variables include dispersal patterns, mating strategies, home-range sizes, body sizes, and social structures.

CHAPTER 5: Results

For the MC1R protein, the amino acid sequence had been previously sequenced for all nine NHP species included in this study. These sequences were obtained from the UniProt data bank. The MC1R amino acid sequence and the corresponding total number of amino acids for each species are presented in Tables 4 and 5, respectively. Coincidentally, all nine NHP species have an amino acid sequence of equal length, 317 amino acids. In contrast, the amino acid sequences for the OCA2 protein has only been sequenced for four of the species of interest, chimpanzees, white-cheeked gibbons, black snub-nosed monkeys, and long-tailed macaques. The OCA2 amino acid sequence and the corresponding total number of amino acids for each species is shown in Table 6 and Table 7.

Table 4. MC1R amino acid sequences for the nine nonhuman primate species of study

Species	Amino Acid Sequence
Chimpanzee *(Pan troglodytes)*	MAVQGSQRRLLGSLNSTPTAIPQLGLAANQTGARCLEVSIPDGLFLSLGLVSLVENMLVVATIAKNRNLHSPMYCFICCLALSDLLVSGSNVLETAVILLLEAGALVARAAVLQQVDNVIDVITCSSMLSSLCFLGAIAVDRYISIFYALRYHSIVTLPRARRAIAAIWVASVLFSTLFIAYCDHTAVLLCLVVFFLAVLVLMAVLYVHMLARACQHAQGIARLHKRQRPVHQGFGLKGAVTLTILLGIFFLCWGPFFLHLTLIVLCPEHPTCGCIFKNFNLFLALIICNAIIDPLIYAFHSQELRRTLKEVLTCSW
Lar Gibbon *(Hylobates lar)*	MAVQGFQRRLLGSLNSTPTAIPQLGLAANQTGARCLEVSIPDGLFLSLGLVSLVENVLVVATIAKNRNLHSPTYCFICCLALSDLLVSGGNVLETVVILLLEASALAARAAVVQPLDNVIDVITCSSMVSSLCFLGAIAMDRYVSIFYALRYHSIVTLPRARQAIAAIWVASVLFSTLFIAYYDHAAVLLCLVVFFLAMLVLMAVLYVHMLARACQHAQGIARLHKRQRPLHQGFGLKGAVTLTILLGIFFLCWGPFFLHLTLIVLCPQHPTCSCIFKNFNLFLTLIICNAIIDPLIYAFRRQELRRTLKEGLTCSW
Black Howler Monkey *(Alouatta caraya)*	MPMQGAQRRLLGSLNSTPTATPNLGLAANHTGAPCLEVSIPHGLFLSLGLVSLVENVLVVAAIAKNRNLHSPMYCFICCLALSDLLVSGSNMLETAVILLLEAGALATRASVVQQLQNTIDVLTCSSMLCSLCFLGAIAVDRYVSIFYALRYHSIVTLPRARRAIAAIWVASVLSSTLFIAYCDHAAVLLCLVVFFLAMLVLMAVLYVHMLARACQHAQGITRLHKRQLPAHQGFGLRGAATLTILLGIFFLCWGPFFLHLMLVVLCPQHLTCSCIFKNFKVFLTLIICNTIIDPLIYAFRSQELCRTLKEVLLCSW
Brown Lemur *(Eulemur fulvus fulvus)*	MPAQGSQRGLLGAVNFTPTATPHLRPAANQTGPQCLEVSVPDGLFLCLGLVSLVENTLVVAAIAKNRNLHSPMYCFICCLALSDLLVSVSNLLETAVLLLLEVGALAAQATVVQQLGNVIDVLICSSMVSSLCSLGAIAMDRYISIFYALRYHSIVTLARARRAIAAVWAASILSSTLFITYYDRTAALLCLVVFFLAMLVLMALLYVHMLIQACQHAQAIARLHKRQHPVQQGWGLKGAATLTILLGVFFLCWGPFFLHLTLIAVCPQHPTCSCIFKNFRLFLALIICNTIVDPLIYAFRSQELRRTLKEVLLFSW

White-cheeked Gibbon *(Nomascus leucogenys)*	MAVQGFQRRLLGSLNSTPTAIPQLRLAANQTGARCLEVSIPDGLFLSLGLVSLVENVLVVAAIAKNRNLHSPTYCFICCLALSDLLVSGGNVLETVVILLLEASALAARAAVVQQLDNVIDVITCSSMVSSLCFLGAIAVDRHVSIFYALRYHSIVTLPRARQAIAAIWVASVLFSALFIAYCDHAAVLLCLVVFFLAMLVLMAVLYVHMLARACEHAQGIAQLHKRQRPLHQGFGLKGAVTLSILLGIFFLCWGPFFLHLTLIVLCPQHPTCSCIFKNFNLFLTLIICNAIIDPLIYAFRRQELRRTLKEVLMCSW
Mantled Howler Monkey *(Alouatta palliata)*	MPMQGAQRRLLGSLNSTPTATPNLGLAANHTGAPCLEVSIPDGLFLSLGLVSLVENVLVVAAIAKNRNLHSPMYCFICCLALSDLLVSGSNMLETAVILLLEAGALATRASVVQQLQNTIDVLTCSSMLCSLCFLGAIAVDRYVSIFYALRYHSIVTLPRARRAIAAIWVASVLSSTLFIAYCDHAAVLLCLVVFFLAMLVLMAVLYVHMLARACQHAQGITRLHKRQLPAHQGFGLRGAATLTILLGIFFLCWGPFFLHLMLVVLCPQHLTCSCIFKNFKVFLTLIICNTIIDPLIYAFRSQELCRTLKEVLLCSW
Mueller’s Gibbon *(Hylobates muelleri)*	MAVQGFQRRLLGSLNSTPTAIPQLGLAANQTGARCLEVSIPDGLFLSLGLVSLVENVLVVATIAKNRNLHSPTYCFICCLALSDLLVSGGNVLETVVILLLEASALAARAAVVQPLDNVIDVITCSSMVSSLCFLGAIAVDRYVSIFYALRYHSIVTLPRARQAIAAIWVASVLFSTLFIAYYDHAAVLLCLVVFFLAMLVXMAVLYVHMLARACQHAQGIARLHKRQRPLHQGFGLKGAVTLTILLGIFFLCWGPFFLHLTLIVLCPQHPTCSCIFKNFNLFLTLIICNAIIDPLIYAFRRQELRRTLKEGLTCSW
Black Snub-nosed Monkey *(Rhinopithecus bieti)*	MPVQGSQRRLLGSLNSTPTATPKLGLAANQTGARCLEVSIPDGLFLSLGLVSLVENVLVVAAIARNRNLHSPMYCFICCLALSDLLVSGSNMLETAVILLLEAGALAARAAVVQQLDNVIDVITCSSMLSSLCFLGAIAVDRYISIFYALRYHSIVTLPRARRVVAAIWVASVLFSTLFIAYYNHAAVLLCLVVFFLAMLVLMAVLYIHMLARACQHAQGIAQLHKRQRPAHQGVGLKGAATLTILLGIFFLCWGPFFLHLTLIVLCPQHPTCSCIFKNFNLFLALIICNTIIDPLIYAFRSQELRRTLKKVLLCSW
Long-tailed Macaque *(Macaca fascicularis)*	MPVQGSQRRLLGSLNSTPTATPHLGLAANQTGARCLEVSIPDGLFLSLGLVSLVENVLVVTAIAKNRNLHSPMYCFICCLALSDLLVSGSNMLETAVTLLLEAGALAARAAVVQQLDNVIDVITCSSMLSSLCFLGAIAVDRYISIFYALRYHSIVTLPRARRAIAAIWVASVLCSTLFIAYYDHAAVLLCLVVFFLAMLVLMAVLYVHMLARACQHAQGIARLHKRQRLAHQGFGLKGAATLTILLGIFFLCWGPFFLHLTLIVLCPQHPTCSCIFKNFNLFLALIICNAIIDPLIYAFRSQELRRTLKEVLLCSW

Table 5. MC1R amino acid sequence length and presence/absence of dichromatism

Species	Sequence Length	Dichromatic Pelage (Yes/No)
Chimpanzee	317	No
Lar Gibbon	317	Yes
Black Howler Monkey	317	Yes
Brown Lemur	317	No
White-cheeked Gibbon	317	Yes
Mantled Howler Monkey	317	No
Mueller’s Gibbon	317	No
Black Snub-nosed Monkey	317	No
Long-tailed Macaque	317	No

Table 6. OCA2 amino acid sequence for the nine nonhuman primate species of study

Species	Amino Acid Sequence
Chimpanzee	MHLEGRDGRRYPGTPAVELLQTSVPTGLAELAAGKHRLPRGAGGADPSHSCPSGAAGQSSWAPAGQEFASFLTKGRSHSSLPQMSSSRSKDSCFTENTPLLRNSLQEKGSRCIPVYHPEFITAEESWEDSSADWERRYLLSREVSGLSASASSEKGDLLDSPHIRLRLSKLRRCVQWLKVTGLFVFVVLCSILFSLYPDQGKLWQLLALSPLENYSVNLSSHVDSTLLQVDLAGALVASGPSRPGREEHIVVELTQADALGSRWRRPQQVTHNWTVYLNPRRSEHSVMSRTFEVLTRETVSISIRASLQQTQAVPLLMAHQYLRGSVEAQVTIATAILAGVYALIIFEIVHRTLAAMLGSLAALAALAVIGDRPSLTHVVEWIDFETLALLFGMMILVAIFSETGFFDYCAVKAYRLSRGRVWAMIIMLCLIAAVLSAFLDNVTTMLLFTPVTIRLCEVLNLDPRQVLIAEVIFTNIGGAATAIGDPPNVIIVSNQELRKMGLDFAGFTAHMFIGICLVLLVCFPLLRLLYWNRKLYNKEPSEIVELKHEIHVWRLTAQRISPASREETAVRRLLLGKVLALEHLLAQRLHTFHRQISQEDKNWETNIQELQKKHRISDGILLAKCLTVLGFAIFMFFLNSFVPGIHLDLGWIAILGAIWLLILADIHDFEIILHRVEWATLLFFAALFVLMEALAHLHLIEYVGEQTALLIKMVPEEQRLTAAIVLVVWVSALASSLIDNIPFTATMIPVLLNLSHDPEVGLPAPPLMYALAFGACLGGNTLI GRDGRQ GASANVVCAGIAEQHGYGFSFMEFFRLGFPMMVVSCTVGMCYLLVAHVVVGWN
White-cheeked Gibbon	MHLEGRDGRQYSGAPAVELLQTSVPTGLAELAAGKHRPPRGAGRADPSHSCPSGTTGQSSWAPAGQEFASFLTKGRSHSPLPQMSSSRSKDSCFTENTPLLRNSLQEKGSRCIPVYHPEFITAEESWEDSSADWERRYLLSREVSGLSASASSEKGDLLDSPHIRLRLSKLRRCVQWLKVTGLFVFVVLCSILFSLYPDQGKLWQLLALSPLENYSMNLSSHVDSTLLQVDLAGALVASGPSRPGREEHIVVELTQADTLGSRWRRPQQVMGGPLTVGLVNSKVIVSFCRETVSISIQASLQQTQAVPLLMAHQYLRGSVEAQVTIATAILAGVYALIIFERPSLTHVVEWIDFETLALLFGMMILVAIFSETGFFDYCAVKAYRLSRGRVWAMIIMLCLIAAVLSAFLDNVTTMLLFTPVTIRYAKHLCRQVLIAEVIFTNIGGAATAIGDPPNVIIVSNQELRKMGLDFAGFTAHMFVGICLVLLVCFPLLRLLYWNRKLYNKEPSEIVELKHEIHVWRLTAQRISPASREETAVRRLLLGKVLALEHLLARRLHTFHRQISQEDKNWETNIQELQKKHRISDGILLAKCLTVLGFVIFMFFLNSFVPGIHLDLGWIAILGAIWLLILADIHDFEIILHRVEWATLLFFAALFVLMEALAHLHLIEYVGEQTALLIKMVPEEQRLTAAIVLVVWVSALASSLIDNIPFTATMIPVLLNLSHDPEVGLPAPPLMYALAFGACLGGNTLIGASANVVCAGIAEQHGYGFSFMEFFRLGFPMMVVSCTVGMCYLLVAHVVVGWN
Black Snub-nosed Monkey	MHLEGRDGRRYPGAPEVELLQTSVPPGLAELAAGKRRPPRGAGGVDPSHSCPSGAAGQSSWAPAGQEFASFLTKGRSHSPLPQMSSSRSKDSCFTENTPLLRNSLQEKGSRCIPVYHPEFITAEESWEDGSADWERRYLLSREVSGLSASASSEKGDLPDSPHVRLRLSKLRCCVQWLKVTGLFVFVVLCSILFSLYPDQGKLWQLLALSPLENYSVNLSSHGDSTLLQVDLAGALVASGPSRSGREERLLVELTQADTSGSRWQRPQQVTHNWTVYLNPRRSERSVMSRTFEVLGRETMSISIRASLQQTQAVPLLMAYQYLRASVEAQVTIATAILAGVYALIIFEVVHRTLAAMLGSLAALAALAVIGDRPSLTHVVEWIDFETLALLFGMMILVAIFSETGFFDYCAVKAYRLSRGRVWAMIIMLCLIAAVLSAFLDNVTTMLLFTPVTIRLCEVLNLDPRQVLIAEVIFTNIGGAATAIGDPPNVIIVSNQELRKMGLDFAGFTAHMFIGICLVLLVSFPLLRLLYWNRKLYNKEPSEIVELKHEIHVWRLTAQRISPASREETAVRRLLLGKVLALEHLLARRLHTFHRQISQEDKNWETNIQELQKKHRISDGILLAKCLTVLGFVIFMFFLNSFVPGIHLDLGWIAILGAIWLLILADIHDFEIILHRVEWATLLFFAALFVLMEALAHLHLIEYVGEQTALLIKMVPEEQRLTAAIVLVVWVSALASSLIDNIPFTATMIPVLLNLSRDPEVGLPAPPLMYALAFGACLGGNTLIGASANVVCAGIAEQHGYGFSFMEFFRLGFPMMIVSCIVGMCYLLVAHVVVGWN
Long-tailed Macaque	MHLEGRDGRRDPGTPEVELLQTSGPPGLAELAAGKRRPPRGAGGADPSLSCPSGAAGQSSWAPAGQEFASFLTKGRSHSPLPQMSSSRSKDSCFTENTPLLRNSLQEKGRCVQWLKVTGLFVFVVLCSILFSLYPDQGKLWQLLALSPLENYSVNLSSHGDSTLLQVDLAGALVASGPSRSGREERLLVELTQADTSGSRWRRPQQVTHNWTVYLNPRKSERSVMSRTFE

	VLGRETVSISIRASLQQTQAVPLLMAYQYLHASVEAQVTIATAILAGVYALIIFEIVHRTLAAMLGSLAALAALAVIGDRPSLTHVVEWIDFETLALLFGMMILVAIFSETGFFDYCAVKAYRLSRGRVWAMIIMLCLIAAVLSAFLDNVTTMLLFTPVTIRLCEVLNLDPRQVLIAEVIFTNIGGAATAIGDPPNVIIVSNQELRKMGLDFAGFTAHMFLGICLVLLVSFPLLRLLYWNRKLYNKEPSEIVELKHEIHVWRLTAQRISPASREETAVRRLLLGKVLALEHLLALRLHTFHRQISQEDKNWETNIQELQKKHRISDGILLAKCLTVLGFVIFMFFLNSFVPGIHLDLGWIAILGAIWLLILADIHDFEIILHRVEWATLLFFAALFVLMEALAHLHLIEYVGEQTALLIKMVPEEQRLTAAIVLVVWVSALASSLIDNIPFTATMIPVLLNLSRDPEVGLPAPPLMYALAFGACLGGNGTLIGASANVVCAGIAEQHGYGFSFMEFFRLGFPMMIVSCIVGMCYLLVAHVVVGWN

Table 7. OCA2 amino acid sequence length and the presence/absence of dichromatism

Species	Sequence Length	Dichromatic Pelage (Yes/No)
Chimpanzee	838	No
White-cheeked Gibbon	804	Yes
Black Snub-nosed Monkey	838	No
Long-tailed Macaque	775	No

MC1R amino acid sequence differences among the nine NHP species of study

The amino acid sequences of each of the nine NHP species, were compared to one another to identify any differences. For the purpose of this study, a difference is defined as any case wherein the amino acids for two species at the same location do not match (i.e. glycine versus glutamine). The differences between the nine species are shown in Table 8. The differences are reported with the actual number of amino acid differences and percentages. These percentages represent how many differences occur out of the total number of amino acids. Between the nine species, no difference exceeded 21%. The largest observed difference was between white-cheeked gibbons and brown lemurs (20.82%) and the smallest observed difference was between the two *Alouatta* species, black howlers and mantled howlers (0.315%). Such small differences suggest that the *MC1R* gene is highly conserved between NHP species, and within this comparison, nine conserved sequences were identified. For the purpose of this study, a conserved sequence is defined as any sequence that is longer than five amino acids and is identical in all the species that are compared. The longest conserved sequence was "YCFICCLALSDLLVS", a sequence of 15 amino acids. All the conserved sequences are presented in Table 9, with their corresponding length.

The differences in the MC1R sequences found here do not reveal a strong distinction between the three NHP species that exhibit dichromatic pelage (*H. lar, A. caraya*, and *N. leucogenys*) versus the six species that do not exhibit dichromatic pelage (*P. troglodytes, E. fulvus*, *A. palliata*, *H. muelleri*, *R. bieti*, and *M. fascicularis*). Furthermore, there was no conserved sequence (>5 amino acids) that existed only within the three dichromatic species. During the comparison, I expected to find lower differences when two dichromatic species were compared. However, that was not the case. For example, the black howler and the lar gibbon has an observed difference of 12.934%, which is the same difference between chimpanzees and black howlers. There were no shared patterns recognized in the MC1R amino acid sequences of the three dichromatic species. In comparison there was a pattern that was recognized between all the nine species. Each species' lowest observed difference occurred when they were compared to either a sister species or the closest evolutionarily related species. For example, the two species of howlers have a difference of 0.315% and the two *Hylobates* species has an observed difference of 0.631%. The white-cheeked gibbon had its lowest observed difference when compared to the other two gibbon species, both of which have an observed difference of 3.785%, as shown in Figure 5.

Table 8. Differences in the percentages and the actual number of amino acids presented in parentheses for each of the MC1R sequences for the nine NHP species compared in this study

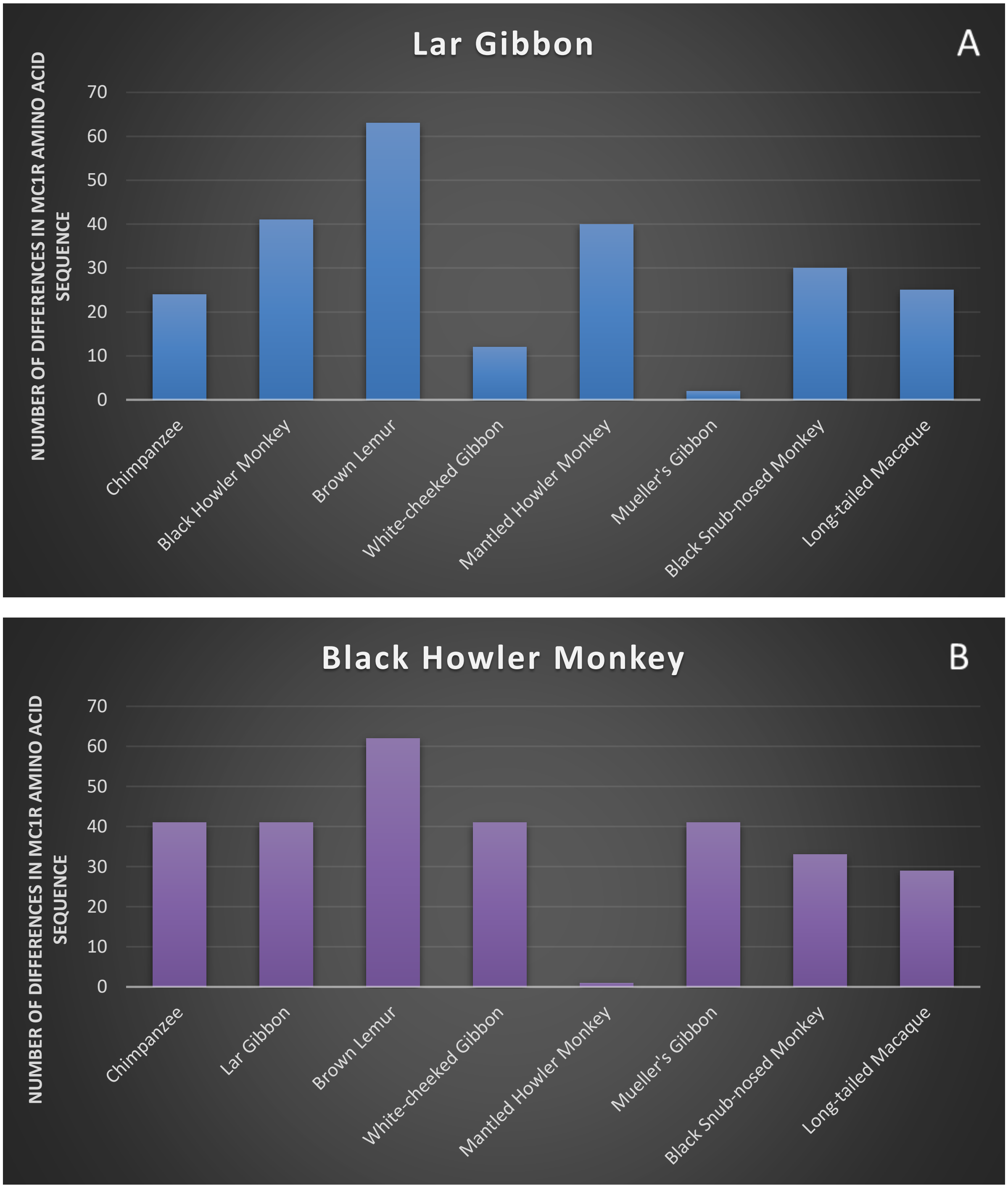
Species	Chimpanzee	Lar Gibbon	Black Howler Monkey	Brown Lemur	White-cheeked Gibbon	Mantled Howler Monkey	Mueller's Gibbon	Black Snub-	Long-tailed Macaque

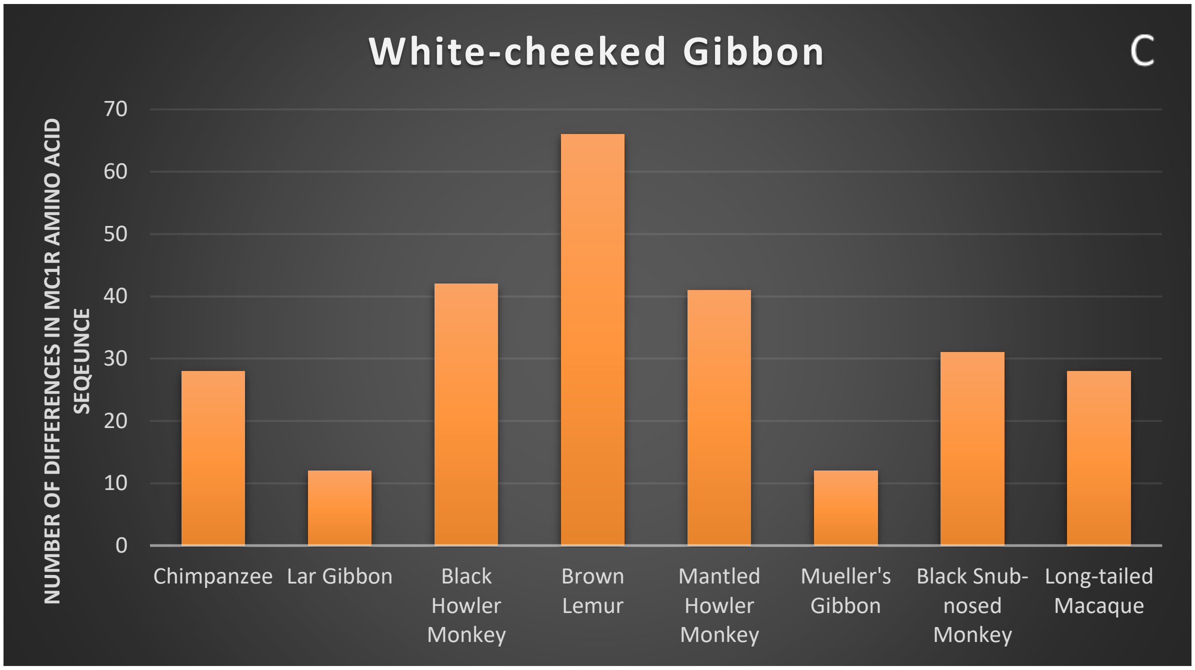
								nosed Monkey	
Chimpanzee		7.571% (24)	12.934% (41)	18.927% (60)	8.833% (28)	12.618% (40)	7.571% (24)	8.517% (27)	6.940% (22)
Lar Gibbon	7.571% (24)		12.934% (41)	19.874% (63)	3.785% (12)	12.618% (40)	0.631% (2)	9.464% (30)	7.886% (25)
Black Howler Monkey	12.934% (41)	12.934% (41)		19.558% (62)	13.249% (42)	0.315% (1)	12.934% (41)	10.411% (33)	9.148% (29)
Brown Lemur	18.927% (60)	19.874% (63)	19.558% (62)		20.820% (66)	19.243% (61)	20.505% (65)	17.350% (55)	15.773% (50)
White-cheeked Gibbon	8.833% (28)	3.785% (12)	13.249% (42)	20.820% (66)		12.934% (41)	3.785% (12)	9.779% (31)	8.833% (28)
Mantled Howler Monkey	12.618% (40)	12.618% (40)	0.315% (1)	19.243% (61)	12.934% (41)		12.618% (40)	10.095% (32)	8.833% (28)
Mueller's Gibbon	7.571% (24)	0.631% (2)	12.934% (41)	20.505% (65)	3.785% (12)	12.618% (40)		9.464% (30)	7.962% (25)
Black Snub-nosed Monkey	8.517% (27)	9.464% (30)	10.411% (33)	17.350% (55)	9.779% (31)	10.095% (32)	9.464% (30)		4.416% (14)
Long-tailed Macaque	6.940% (22)	7.886% (25)	9.148% (29)	15.773% (50)	8.833% (28)	8.833% (28)	7.962% (25)	4.416% (14)	

Table 9. MC1R conserved sequences and length

Conserved Sequence	Sequence Length
CLEVS	5
LGLVSLVEN	9
NRNLHSP	7
YCFICCLALSDLLVS	15
YALRYHSIVTL	11
LLCLVVFFLA	10
GPFFLHL	7
IFKNF	5
DPLIYAF	7

Figure 5. Observed differences in MC1R amino acid sequences of the sexually dichromatic Lar gibbon (A), black howler monkey (B), and White-cheeked gibbon (C) compared to non-sexually dichromatic species of nonhuman primates





OCA2 amino acid sequence differences among the four NHP species of study

After obtaining the OCA2 amino acid sequence from each of the four available NHP species (chimpanzees, white-cheeked gibbons, black snub-nosed monkeys, and long-tailed macaques) from Uni Port, they were compared using the same methods for the MC1R sequences. The differences between the four species are shown in Table 10. The differences are reported with the actual number of amino acid differences and percentages. These percentages represent the number of differences that occurred among the total number of amino acids in the sequence. The number of amino acids varied for the OCA2 sequence (838, 804, and 775). To account for different number of sequences, I altered each NHP sequence to match in length (see description in Methods). The removed amino acid sequences can be found in Table 11. There were three species that required a reduction in their MC1R amino acid sequence (chimpanzee, white-cheeked gibbon, and black snub-nosed monkey) and Table 11 shows that the same nucleotides were removed from the amino acid sequences to either match the amino acid length of 804 (white-cheeked gibbon) or 775 (long-tailed macaque). No differences exceeded 38% among the four species. The largest observed difference was between the white-cheeked gibbon and the black snub-nosed monkey (37.548%), and the smallest difference was between the black snub-nosed monkey and the long-tailed macaque (1.677%). The small differences found here suggest that the *OCA2* gene is highly conserved between NHP species. Overall, there were 22 conserved sequences between the four OCA2 sequences. The longest conserved sequence was:

"IFMFFLNSFVPGIHLDLGWIAILGAIWLLILADIHDFEII-LHRVEWATLLFFAALFVLMEALAHLHLIEYVGEQTALLIKMVPEEQRLTAAIVLVVWVSALASSLIDNIPFTATMIPVLLNLS"

This sequence consists of 123 amino acids. There were more conserved sequences within the OCA2 sequences compared to MC1R. Furthermore, the length of the conserved sequences of OCA2 are much longer. All the conserved sequences are presented in Table 12 with their corresponding length.

Among these four NHP species, the only dichromatic species is the white-cheeked gibbon. The largest differences in the sequences was between the white-cheeked gibbon and long-tailed macaque comparison (37.584%). All other sequence differences with the macaque were below 4%. Furthermore, the highest observed differences for each monochromatic species (chimpanzee, black snub-nosed monkey, long-tailed macaque) emerged when they were compared to the dichromatic white-cheeked gibbon. After these comparisons, there were three unique sequences present in white-cheeked gibbons. For the purpose of this study, a unique sequence is defined as a sequence where a minimum of five amino acids that are unique to only one species. The sequences that are unique to the white-cheeked gibbon are presented in Table 13.

Table 10. Differences in the percentages and the actual number of amino acids presented in parentheses for each of the OCA2 sequences for the four NHP species compared in this study

Species	Chimpanzee	White-cheeked Gibbon	Black Snub-nosed Monkey	Long-tailed Macaque
Chimpanzee		4.736% (38)	3.699% (31)	3.613% (28)
White-cheeked Gibbon	4.736% (38)		6.468% (52)	37.548% (291)
Black Snub-nosed Monkey	3.699% (31)	6.468% (52)		1.677% (13)
Long-tailed Macaque	3.613% (28)	37.548% (291)	1.677% (13)	

Table 11. Amino acid sequences (and its location) removed from the OCA2 sequences of the four NHP species

Sequence	**Location in Sequence**	**Species**	**Remaining Sequence Length**
TFEVLTR	291-297	Chimpanzee	804
IVHRTLAAMLGSLAALAALAVIGD	349-372	Chimpanzee	804
NLD	461-463	Chimpanzee	804
SRCIPVYHPEFITAEESWEDSSADWERRYLLSREVSGLSASASSEKGDLLDSPHIRLRLSKLR	110-172	Chimpanzee	775
SRCIPVYHPEFITAEESWEDSSADWERRY	110-138	White-cheeked Gibbon	775
THNWTVY	271-277	Black Snub-nosed Monkey	804
VVHRTLAAMLGSLAALAALAVIGD	349-372	Black Snub-nosed Monkey	804
NLD	461-463	Black Snub-nosed Monkey	804
SRCIPVYHPEFITAEESWEDGSADWERRYLLSREVSGLSASASSEKGDLPDSPHVRLRLSKLR	110-172	Black Snub-nosed Monkey	775

Table 12. OCA2 conserved sequences and length

Conserved Sequence	**Length**
MHLEGRDGR	9
VELLQTS	7
GLAELAAGK	9
PRGAG	5
SCPSG	5

SSWAPAGQEFASFLTKGRSHS	21
LPQMSSSRSKDSCFTENTPLLRNSLQEKG	29
CVQWLKVTGLFVFVVLCSILFSLYPDQGKLWQLLALSPLENYS	43
NLSSH	5
DSTLLQVDLAGALVASGPSR	20
VELTQAD	7
ASLQQTQAVPLLMA	14
EAQVTIATAILAGVYALIIFE	21
PSLTHVVEWIDFETLALLFGMMILVAIFSETGFFDYCAVKAYRLSRGRVWAMIIMLCLIAAVLSAFLDNVTTMLLFTPVTIR	83
QVLIAEVIFTNIGGAATAIGDPPNVIIVSNQELRKMGLDFAGFTAHMF	48
GICLVLLV	8
FPLLRLLYWNRKLYNKEPSEIVELKHEIHVWRLTAQRISPASREETAVRRLLLGKVLALEHLLA	64
RLHTFHRQISQEDKNWETNIQELQKKHRISDGILLAKCLTVLGF	44
IFMFFLNSFVPGIHLDLGWIAILGAIWLLILADIHDFEIILHRVEWATLLFFAALFVLMEALAHLHLIEYVGEQTALLIKMVPEEQRLTAAIVLVVWVSALASSLIDNIPFTATMIPVLLNLS	123
DPEVGLPAPPLMYALAFGACLGGNGTLIG	29
SANVVCAGIAEQHGYGFSFMEFFRLGFPMM	30
VGMCYLLVAHVVVGWN	16

Table 13. Unique OCA2 amino acid sequences and position of the white-cheeked gibbon

Sequences	**Amino Acid Position**
GRDGQ	5-10
MGGPLTVGLVNSKVIVSFC	271-289
YAKHLC	425-430

Differences in socioecological variables for each NHP species

To further expand upon these genetic data, I also compared socioecological variables for each of the nine NHPs. For the purpose of this study, I focused on those variables (dispersal pattern, home-range size, group size, and mating patterns) that would potentially play a strong evolutionary force that would select for sexual dichromatism. For comparative purposes, I also included these variables for the sister species of the dichromatic species. For each socioecological variable, there was a large degree of variation among the nine NHP species. For example, home-range size varies from 3.6 ha (black howlers) to ~ 2200 ha (black snub-nosed monkeys). Figure 6 shows the home-range size of each species. The figure illuminates a noticeable pattern, all dichromatic species have smaller home-ranges when compared to a monochromatic sister species. The three dichromatic species varied with mating patterns, both gibbon species are socially monogamous and the black howler monkey exhibits polygynandrous/ polygynous mating patterns. Furthermore, the black howler monkey is sexual dimorphic, but the lar gibbon and white-cheeked gibbon are both sexually monomorphic. The remaining socioecological variables of the nine NHP species are presented in Table 14.

Figure 6. Home-range (hectares) of the nine NHP species (3D bars denote dichromatic species)

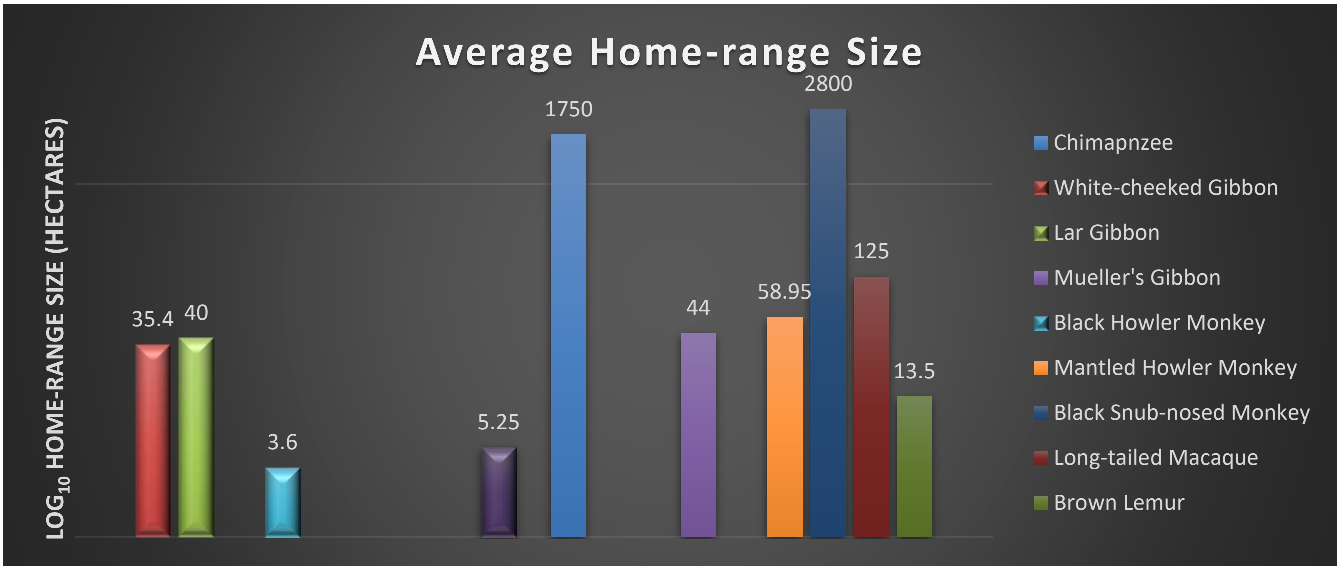


Table 14. Socioecological variables of the nine NHP species of study

Species	Dichromatic Pelage	Mating Pattern	Sexual Morphism	Dispersal Pattern	Average Group Size	Sources
Chimpanzee	No	Polygynandrous/ Polygynous	Dimorphic	Female	20-150	Smith and Jungers, 1997; Boesch and Lehmann, 2003; Pusey, 1979

Lar Gibbon	Yes	Monogamous	Monomorphic	Male and female	2-5	Bartlett, 2007; Groves, 1972, 1984; Gron, 2010
Black Howler Monkey	Yes	Polygynandrous/ Polygynous	Dimorphic	Male and female	10-15	Bravo and Sallenave, 2003; Zunino and Rumiz, 1986; Glander, 1978; Chapman, 1988).
Brown Lemur	No	Unknown	Monomorphic	Male and female*	12	Harrington, 1975; Ganzhorn, 1988; Mittermeier, 1994; Colquhoun, 1993; Andrews and Birkinshaw, 1998
White-cheeked Gibbon	Yes	Monogamous	Monomorphic	Male and female	2-5	Bartlett, 2007; Groves, 1972, 1984
Mantled Howler Monkey	No	Polygynandrous	Dimorphic	Female	10-20	Bravo and Sallenave, 2003; Zunino and Rumiz, 1986; Glander, 1978; Chapman, 1988).
Mueller's Gibbon	No	Monogamous	Monomorphic	Male and female	2-5	Bartlett, 2007; Groves, 1972, 1984
Black Snub-nosed Monkey	No	Polygynous	Dimorphic	Female*	15-70	Kirkpatrick et al, 1998

Long-tailed Macaque	No	Polygynandrous/ Polygynous	Dimorphic	Male	30	Smith and Jungers, 1997**;** Dittus 2004

*Poorly documented

CHAPTER 6: Discussion and conclusions

Sexual dichromatism is a rare trait and occurs in less than 1% of nonhuman primate species. It is a puzzling trait given that it occurs among these different taxa in nonhuman primates (lemurs, Neotropical monkeys, and lesser apes). Although no study has systematically examined dichromatism in nonhuman primates (NHPs), others examined the importance of neonatal coats, sexual selective coloration (faces, pelage), such as the colored patches of skin on the chests of male geladas, and the adaptability of fur coloration because these traits are found more frequently across many primate lineages (Bergman and Beehner, 2009). Furthermore, these traits often fit nicely into current models of primate socioecology. In contrast, a genetic and socioenvironmental basis for sexual dichromatism is currently not well understood or fit well into conventional models to understand primate biology and behavior. To address this gap, this thesis combines genetics and socioecological data together to provide some insights into the origins of dichromatism and the adaptability of sexual dichromatism.

The differences in MC1R sequences between the nine NHP species

This project initially set forth to examine the genetic mechanism(s) behind sexual dichromatism. I analyzed and compared the MC1R amino acid sequences for nine NHPs. Although the *MC1R* gene is understood to regulate pigmentation in humans, these results of this study suggest that the *MC1R* gene does not play a prominent role in regulating NHP sexual dichromatism as evidenced by the fact that there were no conserved sequences found within the three dichromatic species (i.e. the black howler monkey [*Alouatta caraya*], lar gibbon [*Hylobates lar*], and white-cheeked gibbon [*Nomascus leucogenys*]). Furthermore, the differences in amino acid sequences between the NHP species that exhibit dichromatic pelage were higher than the differences between two sister species. For example, the black howler monkey's MC1R amino acid sequence varied from the lar gibbon by 12.934%, but only varies 0.315% from the mantled howler monkey (*Alouatta palliata*). These results suggest that the *MC1R* gene and consequently, its amino acid sequence, exhibit a high phylogenetic signal. A high phylogenetic signal occurs when close relatives resemble each other more than they resemble distant relatives. In contrast, a low phylogenetic signal occurs when a species resembles closely related species as much as they resemble a more distant species (Kamilar and Cooper, 2013). This is true for eight of the nine NHP species that were analyzed. However, this pattern was not observed for the long-tailed macaque (*Macaca fascicularis*). For example, the MC1R amino acid sequence of the chimpanzee (*Pan troglodytes*) varies from the two hylobatids (the lar and white-cheeked gibbons) by 7.571% but varies from the long-tailed macaque by only 6.940%. This contradicts the pattern of high phylogenetic signal since the two gibbon species are phylogenetically closer to the chimpanzee than the long-tailed macaque. This difference can be a result of a multitude of reasons, such as a mutation that occurred among long-tailed macaques after they split from a common ancestor with apes or it is possible that the long-tailed macaque may have different selective forces acting on its MC1R amino acid sequence and is a product of convergent evolution. So, although the *MC1R* gene does not appear to be directly linked to sexually dichromatic pelage it would be naive to completely rule out the importance of *MC1R*. It is possible that the *MC1R* gene may play an indirect role in regulation through a multitude of genetic processes such as genetic hitchhiking, a pattern wherein genes are linked, but there is strong selection pressure on only one of those genes. This phenomenon could explain how all the genes that are linked and would benefit from the positive selection pressure (Kim and Stephan, 2002).

The differences in OCA2 sequences between the four NHP species

The *OCA2* gene was compared between only four of the nine species of this study, chimpanzees, black snub-nosed monkeys (*Rhinopithecus bieti*), long-tailed macaques, and white-cheeked gibbons. Unlike the *MC1R* comparison, it is important to note that there is only one dichromatic NHP species included in this comparison, the white-cheeked gibbon. These data suggest that the *OCA2* gene may play an important role in regulating sexually dichromatic pelage. Not only were there multiple unique sequences found in the white-cheeked gibbon's amino acid sequence (Table 13), there are 22 conserved sequences between these four NHP species (Table 12). This suggest that the *OCA2* gene is an evolutionary conserved gene which throughout a relatively long evolutionary timeframe has essentially remained unchanged. This pattern indicates that the *OCA2* gene is both unique and essential. Furthermore, since the gene is highly conserved, changes in the gene are likely to be drastic and/or deleterious. In addition to the highly conserved sequence of the gene, the comparison of the OCA2 amino acid sequence revealed an important pattern, all the species compared experience their highest level of variance when compared to the white-cheeked gibbon. The amino sequence of the chimpanzee varied from the black snub-nosed monkey and the long-tailed macaque by 3.699% and 3.623%, respectively, but varied by 4.736% from the white cheeked gibbon. The black snub-nosed monkey exhibits a similar pattern, it varied less than 4% from the chimpanzee and long-tailed macaque but varied by more than 6% compared to the white-cheeked gibbon. This pattern is exhibited at a much larger magnitude with the long-tailed macaque, it varied by less than 4% with the chimpanzee and black snub-nosed monkey but varied by about 38% from the white-cheeked gibbon, more than 14 times higher than the average variance between the other three NHP species. Unlike the *MC1R* gene, these data indicate that the *OCA2* gene has a very low phylogenetic signal. As previously stated, it is important to explicitly note that these results and inferences are based off a comparison that included only one dichromatic NHP species. At present, OCA2 genetic data only exists for the white-cheeked gibbon, thus highlighting the need for research on the function and genetic mechanisms of NHP pelage coloration.

Socioecological differences among the NHP study species

This project also analyzed and compared multiple socioecological variables including mating patterns and dispersal patterns, with the intention of illuminating any possible relationships between these variables and sexually dichromatic pelage. My results suggest that both the mating patterns of these NHP species and sexual dimorphism do not have a clear relationship with sexually dichromatic pelage. For the three dichromatic species, the black howler monkey, lar gibbon, and white-cheeked gibbon, the mating patterns are polygynandrous and monogamous, respectively. These two mating patterns are opposites, suggesting that a NHP species' mating pattern did not influence positive selection for sexually dichromatic pelage. Furthermore, although the two gibbon species are both monomorphic, the black howler monkey is a sexually dimorphic species, wherein the males are on average 1.48 times larger than females. These results suggest that neither sexual dimorphism or monomorphism played a significant role in selecting for sexually dichromatic pelage. In contrast, my results did suggest that both dispersal patterns and average home-range size may have a relationship with sexually dichromatic pelage. All three dichromatic species exhibit dispersal patterns in which males and females leave their natal groups suggesting that this dispersal pattern may have led to positive selection for sexually dichromatic pelage, although this sample size is small. Furthermore, average home-range size may also be linked with sexually dichromatic pelage. When comparing home-range sizes, my results demonstrate that all three dichromatic species have smaller average home-range sizes compared to their monochromatic sister-species. For example, the home-range size for black howler monkeys is 1.7 to 5.5 hectares while the range for its monochromatic sister species, the mantled howler monkey, is 9.9 to 108 hectares. A similar pattern is exhibited amongst some lineages of lemurs. The monochromatic brown lemur (*Eulemur fulvus*) has a home-range size of 7 to 20 hectares while its dichromatic sister species, the blue-eyed black lemur's (*E. macaco*) home-range size ranges from 3.5 to 7 hectares. This pattern is also exhibited among the three gibbon species included in this project. These findings suggest that a smaller home-range may have led to positive selection for sexually dichromatic pelage. Smaller home-ranges results in individuals living in close proximity, including sexually mature emigrants, which could cause an increase of mate competition. This relationship could also be a result of functionality of sexually dichromatic pelage. It is possible that dichromatic pelage may serve as camouflage for infants, since in most dichromatic species such as the black howler monkey, the infants exhibit the same pelage color as females. This camouflage may help ensure infant survival by hiding infants upon the mother's coat.

Future implications

As previously mentioned, the data on NHP pelage functionality is currently understudied. This lack of NHP data does not only prevent further understanding of pelage coloration in NHP species, but it also results in a poor comparative framework to understand human hair variation and skin color. To move forward, future research directed at understanding NHP pelage coloration requires sequencing more NHP genomes and publishing their sequences on free online platforms. To date, the most complete lemur genome published thus far is the blue-eyed black lemur, of which is only 79% complete (Meyer et al, 2015). Therefore, in order to further understand the evolution and adaptability of sexually dichromatic pelage, it is essential to have more complete genomes in the future. Towards this end, more NHP amino acid sequences will hopefully be published in the near future, representing more primate clades. Upon examining previously sequenced genes and amino acid sequences, I noticed a bias towards the Great Apes, in particular chimpanzees. This was followed by species of Old World monkeys. This bias needs to be removed, not only to further our understanding of those NHP species with little data, but also to allow for better comparisons across the Order Primates.

This project was limited by both time constraints and funding but acts as a starting point to help shed light onto both the genetic mechanisms underlying sexually dichromatic pelage and the socioecological variables that may have selected for this rare adaptation. Provided I had more funding and time, I would have collected multiple samples, from different individuals, for each of the nine NHP species of interest and then sequenced the OCA2 and MC1R sequences for each sample. This approach would have resulted in genetic heterozygosity within each species, but it would have also provided the possibility to examine if there were any differences in the amino acid sequences between males and females of the same species. Therefore, if there were any differences in the amino acid sequences between males and females of the same species it would be highly likely that the differing amino acid sequences play an important role in regulating dichromatic pelage.

Based on background research, there are a few genes related to pigmentation and melanocyte regulation that should be compared in the future to investigate their roles in regulating sexually dichromatic pelage in nonhuman primates. These genes include the ASIP, TYR, and MITF genes. The ASIP gene deactivates melanocytes, which leads to the production of pheomelanin. The TYR gene is responsible for making tyrosinase, an enzyme located with melanocytes, that is linked to the initial steps of melanin production. Lastly, the MITF gene plays an important role in regulating melanocyte differentiation, proliferation, and survival (Suzuki, 2013; Meyer et al, 2015).

Besides analyzing and comparing more genes that are related to melanin production, it is also important to consider other genetic processes and mechanisms that may play a role in regulating dichromatic pelage. For example, when conducting background research, my initial goal was to examine base-pairs within the genomes of NHP species of interest. However, it was noted that the genomes were not directly related to the amino acid sequences, suggesting that epigenetic factors are probably influencing certain portions of the genome. This study also did not take into consideration the possibility of a founder effect or genetic bottleneck. Bottlenecking occurs when a population's size is reduced after at least one generation, which could therefore decrease the genetic variation within a population (Leberg, 1992). Similarly, the founder effect occurs when a new population is started by a few members of an original, larger population, which also leads to reduced genetic variation (Leberg, 1992). Most NHP species have experienced decreases in population sizes as result of anthropogenic pressures on NHP and their habitats. Evidence suggests that about 60% of NHP species are now threatened with extinction and about 75% have declining populations (Estrada et al, 2017).

Moving forward, sequencing more NHP species across multiple genera, with replications for each species and taking into consideration factors such as, a multitude of genes that regulate melanocytes, the sex of individuals, and other genetic mechanisms and phenomena would build upon what is currently understood about primate pelage.

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