**CONSERVATION GENETICS OF CONFLICT IN THE
ASIAN ELEPHANT, *ELEPHAS MAXIMUS***

A Dissertation

presented to

the Faculty of the Graduate School
at the University of Missouri - Columbia

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

by

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July 2021

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DEDICATIONS

First and certainly most of all, I need to thank my wonderful husband Spencer Budd and our dogs Hogan, Mia, Nigel, and Skippy for moving across the country with me to start this journey and for their daily comfort during the worst days. To Joe Gunn, thank you for enduring this process with me, competing with and pushing me further than anyone, and yet never giving up on either of us. Thank you to my family for their continued support: my Mother, Lenny, and Step-Doug; Randi Lynn and Bryson Phillips, Jackson, and Logan Rasmussen; Jenny and Barry Budd; Abby, Morgan and Emmett Steiner; Sara, Alex, and Finley Horrocks; and Sophie Budd. To Lance Gritton, who steered me headfirst into Biology and reminds me why I began this trip in the first place. My undergraduate mentor Laurie Mauger with the help of Betsy Bancroft, Nathan Werner, and Ron Rodriguez, who went above and beyond to prepare me for this process. My friends at MO Wiggle Butts dog rescue, for keeping me sane and providing an escape when I needed it most. Lastly, to my father, Randell Ranson, who taught me to strive to achieve perfection and for giving rise to my stubborn, always-questioning, rebellious spirit. I hope this would have made you proud.

For Dad and Step-Doug.

ACKNOWLEDGEMENTS

I wish to thank my adviser, Dr. Lori Eggert, for being my mentor throughout these years and never giving up on me and my mad schemes. I would not have accomplished this without Joe Gunn and the rest of the Eggert lab: Jake Burkhardt, Chelsea Titus, Molly Garrett, and Kathryn Storey and the trailblazers for the elephant work, Marissa Ahlering and Stephanie Schuttler. Thank you to my committee, Rex Cocroft, Libby King, and Matt Gompfer, for always being supportive and insightful toward my ideas. I also wish to thank my unofficial committee members, Chris Pires and Lauren Sullivan and their respective lab members, for their welcoming attitude as well as their broad knowledge and innovative thinking. Thank you to Breanne Meyer, Nila Emerich, Nick Valentine, Alan Marshall, Melody Kroll, and Rebecca Ballew for your always needed technical support. This work would not have been possible without the help from my collaborators; Simon Hedges, Martin Tyson, Daophone Suddychanh, Camille Coudrat, Thong Eth Phayvanh, Axay Vongkhamso, Kaoboun Kue, the Nakai-Nam Theun Watershed Management Protection Agency, Nam Theun Power Company, Lao Department of Forestry and Agriculture, and the remaining teams in Laos for all their help in the field. Thank you to Christi Sampson, David Tonkyn, and Peter Leimgruber for inviting me to collaborate on the Bago Yoma project. This research was funded by the National Science Foundation Graduate Research Fellowship Program (NSF GRFP), American Philosophical Society, Sigma Xi, the American Society of Mammalogists, Trans World Airlines Scholarship Program, and the University of Missouri.

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LIST OF ABBREVIATIONS AND ACRONYMS

A	Allelic Diversity
ANOVA	Analysis of Variance
A _P	Private Allelic Richness
A _R	Allelic Richness
AR	Allelic Diversity Ratio
AR _{SE}	Allelic Diversity Ratio Standard Error
BLAST	Basic Local Alignment Search Tool
CITES	Convention on International Trade in Endangered Species
CMR	Capture-Mark-Recapture
DAFO	District Agriculture and Forestry Office
DNA	Deoxyribonucleic Acid
F _{IS}	Inbreeding Coefficient
F _{ST}	Fixation Index, Nuclear Genetic Differentiation
G3	“group of three” elephants
GBS	Genotype by Sequencing
<i>h</i>	MtDNA Haplotype Diversity
H _E	Expected Heterozygosity
H _O	Observed Heterozygosity
HEC	Human-Elephant Conflict
HR	Expected Heterozygosity Ratio
HR _{SE}	Expected Heterozygosity Ratio Standard Error

HWE	Hardy-Weinberg Equilibrium
I	Shannon's Information Index
IUCN	International Union for Conservation of Nature
Lao PDR	People's Democratic Republic of Lao, Laos
MONREC	Ministry of Natural Resources and Environmental Conservation
MtDNA	Mitochondrial Deoxyribonucleic Acid
N	Population Size
NT	Nam Theun
NNT NP	Nakai-Nam Theun National Park
NT2	Nam Theun 2 Hydroelectric Dam
NTPC	Nam Theun 2 Power Company
PCR	Polymerase Chain Reaction
pID	Probability of Identity
PXH NPA	Phou Xang He National Protected Area
pSibs	Probability of Identity for Siblings
QCB	Queen's College Buffer
r	Queller and Goodnight Relatedness
SNP	Single Nucleotide Polymorphism
WCS	Wildlife Conservation Society
WMPA	Watershed Management and Protection Authority
π	MtDNA Nucleotide Diversity
ϕ_{ST}	MtDNA Genetic Differentiation

ABSTRACT

The Asian elephant (*Elephas maximus*) is an endangered species whose distribution spans 13 countries in south, southeast, and insular Asia. The primary threats to the survival of this species include direct conflict, primarily in the forms of poaching and crop-raiding, as well indirect conflict such as habitat loss and fragmentation. Due to the elephant's elusive behavior within their dense vegetative habitat and the fact that their large size presents handling dangers to researchers and elephants, research increasingly relies on noninvasive monitoring combined with a diverse assemblage of genetic tools. This dissertation uses conservation genetics to evaluate major conflict and conservation issues for Asian elephants. The first study, in the Bago Yoma region of Myanmar, evaluates the impact of direct human-conflict in a high-density area of humans and elephants. Here elephants are heavily impacted by the developing skin trade, and condensed populations frequently raid local farms for crops. The study determined the overall population structure and gained demographic insights as to what defines a crop-raider. The second study, conducted in the Nakai plateau elephant population of Lao PDR, compared and contrasted the diversity and demography of a population of high conservation value before and after the construction of a hydroelectric dam. The results revealed a seasonally shifting population, unique from the previous occupants of the plateau, and a decline in genetic diversity. In the final study, genetic data from across the species' range was used to identify hotspots of genetic diversity despite marker selection bias, a problem frequently encountered in conservation genetic studies. The results highlight the evolutionary distinctiveness and conservation value of populations, particularly in southeast Asia, for conservation management of this iconic species.

CHAPTER 1

AN INTRODUCTION TO USING GENETICS IN THE CONSERVATION OF THE ASIAN ELEPHANT

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Published portions in Ahlering, MA, **Budd K**, Schuttler S, Eggert LS. 2020. Genetic analyses of noninvasively collected samples aids in the conservation of elephants. In, Conservation Genomics in Mammals: Integrative research using novel approaches (Ortega, J. and J. E. Maldonado, Eds.). Springer, ISBN 978-3-030-33333-1

KEY THREATS TO THE CONSERVATION OF THE ASIAN ELEPHANT

While Asian elephants have important cultural and religious significance in nearly all 13 countries across their distribution, the increasing urbanization and high human densities of Asia continue to place elephants and humans at odds with one another. Expanding human populations and the recent conversion of habitat for anthropogenic uses have resulted in the compression of many elephant populations into isolated parks and protected areas, few of which are large enough to maintain viable populations over the long-term (Armbruster and Lande 1993). Therefore, elephants are more frequently found in human-dominated landscapes outside and between protected areas, creating the

potential for serious economic and ecological impacts, with tragic consequences for both humans and elephants (Tchamba 1996; Fernando et al. 2005; Kioko et al. 2006; Lee et al. 2006). Human-elephant conflict plays a significant role in management and conservation concerns as human and elephant deaths continue to rise throughout the Asian elephant range (Shaffer et al. 2019). Human-elephant conflict can occur at widespread or local scales through direct contact (i.e., poaching, destruction of property, and crop-raiding) or through indirect contact from anthropogenic land conversion resulting in the displacement of elephant populations (i.e., habitat loss and fragmentation, depleting resources).

Poaching – Poaching for ivory from Asian elephants is a rampant and widespread problem throughout the range, but was likely at its greatest during the twenty-year period spanning 1975-1995 (Sukumar et al. 1998). In Asian elephants, unlike their African counterparts, only males produce tusks. Therefore, severe poaching for ivory can rapidly skew sex ratios. For instance, the male to female ratio was 1:6 in the Indian population of Periyar in 1969 (Kurup 1971), but following severe poaching the ratio increased to 1:122 by 1987-1989 (Chandran 1990). As an adaptive response to high rates of poaching, selection pressures can shift male populations toward becoming tuskless. Although tusklessness was once believed to be relatively rare, as tusked males are thought to have a natural advantage over tuskless males in sexual selection (Watve and Sukumar 1997), many of the surviving males in heavily poached populations are now tuskless (> 90% in Sri Lanka; Kurt 1974; Sukumar 1989). With the loss of males, this massive selection pressure from human conflict has decreased effective population sizes and the majority of

the species' genetic diversity can reside in female lineages in heavily poached populations (Kurt et al. 1995; De Silva et al. 2011; Raubenheimer and Miniggio 2016).

Following strict regulation against the ivory trade across Asia, another poaching crisis has since developed in areas such as Myanmar. Surveys of morning markets along the Chinese border often reveal demands in illegal wildlife products such as pangolin scales (Nijman et al. 2016), Asiatic black bear and sun bear parts (Shepherd and Nijman 2008a), rhinoceros' horn (Shepherd et al. 2018), and elephant products (Nijman et al. 2014). In addition to ivory, in the early 2000s market surveys began to detect an increasing demand for elephant skin, genitalia, and other body parts for what was marketed as traditional medicine; however, no authentic indications of their historical use in traditional eastern medicine has been found. Market dealers also claimed that “elephants were not killed to supply this industry” and that products were being sourced from already deceased elephants found in forest reserves and government-owned logging operations (Shepherd and Nijman 2008b). The validity of such claims was questioned as five independent elephant skin seizures in China during the same timeframe accounted for at least 300 wild elephants (Nooren and Claridge 2001; O'Connell-Rodwell and Parry-Jones 2002; Nijman and Shepherd 2014). In 2006, market surveys found only four pieces of elephant skin, but surveys in 2009 found 278 pieces, suggesting increasing demand. Further support for this increase came from surveys in 2013, which found 1,238 pieces (Nijman and Shepherd 2014). Wildlife organizations such as the World Wildlife Fund and TRAFFIC called for immediate international support in 2017, and started conservation campaigns to raise awareness, combat the skin trade, and prevent further population declines (Kronholm 2017).

Habitat Fragmentation - The Asian elephant is a globally recognized IUCN Red List species and CITES Appendix 1 protected species, yet only 16% of its geographic range is protected (Duckworth and Hedges 1998; Choudhury et al. 2008). Asian elephants, much like African elephants (*Loxodonta africana* and *L. cyclotis*), require large home ranges of relatively homogeneous habitat that, if fragmented, can place them at increased risk for genetic isolation, dispersal limitation, and population decline (Joshi and Singh 2009). In contrast to many other large mammals, elephants can be fairly adaptable to diverse landscapes (Sukumar 2003), however, expanding human populations along with agricultural land conversion and deforestation have heavily fragmented and isolated current elephant populations (Leimgruber et al. 2003). Populations occupying heavily fragmented landscapes are characterized by some of the highest levels of human-elephant conflict and deaths among both humans and elephants (Sukumar 1989; Leimgruber et al. 2003). With restricted habitat, Asian elephants are more likely to participate in crop-raiding behavior to access necessary nutritional resources (Sukumar and Gadgil 1988; Sukumar 1991; Santiapilli and Ramono 1993; Williams et al. 2001; Leimgruber et al. 2003) placing them at additional risk of conflict with humans. The largest unfragmented wildlands for Asian elephants are found in southeast Asia, particularly in Myanmar; however, elephant populations may not occupy these areas (Leimgruber et al. 2003) as connectivity between unoccupied habitat and established populations can be severely limited (He et al. 2020).

USING GENETICS TO EVALUATE CONSERVATION ISSUES IN ASIAN ELEPHANTS

Genetic studies can provide insights into population sizes as well as characteristics of social organization and demography that would have required years of field studies to acquire (Frankham 2010). Traditional genetic studies require blood or fresh tissue, making it particularly difficult to apply genetics to investigations of dangerous or elusive species such as elephants. The development of methods for obtaining DNA from non-invasively collected samples, such as through the collection of elephant feces, has enabled biologists to gain important insights at the individual and population levels (Kohn et al. 1999). Genetic studies also allow for accurate population censusing for elusive species. In a direct comparison between a fecal DNA capture-mark-recapture (CMR) population survey and a traditional dung count, Hedges et al. (2013) found that the fecal DNA CMR methods are more cost-effective and produce comparable, yet more precise, population size estimates along with data on demography and population structure.

Mitochondrial DNA – The most commonly used genetic marker in previous studies of Asian elephants is mitochondrial DNA (mtDNA). This maternally inherited molecule mutates separately from the nuclear genome and is especially suitable for studying species with female-based philopatry, such as elephants (Fernando et al. 2000). Asian elephant studies analyze a mtDNA fragment that spans 630 bp of the C terminal of cytochrome b, threonine and proline tRNAs and the 5' end of the noncoding control region (Fernando et al. 2000). This fragment has been utilized in nearly every Asian elephant genetic study after its publication and is comparable to studies that relied on

previous bovine primers (Fleischer et al. 2001). Additional primers have been developed to sequence the same fragment as Fernando et al. (2000) in heavily degraded samples by amplifying two overlapping regions (Eggert and Budd 2017). In Asian elephants, mtDNA diverged into two separate evolutionary clades approximately 2.5-3.5 million years ago; the α and β clades in Fernando et al. (2000) correspond to clade B and clade A in Fleischer et al. (2001). Using the Fernando et al. (2000) naming convention, the α clade is more frequently found in southeast Asia, while the β is located in south and insular Asia with overlap in clade haplotypes in Myanmar and Thailand (Fernando et al. 2000; Fleischer et al. 2001; Vidya et al. 2009).

Microsatellites – For detecting the effects of contemporary events, studies of Asian elephants, like those of many species of conservation concern, have been heavily reliant on the use of nuclear DNA microsatellite markers. These loci span the Asian elephant genome, but in most conservation genetic studies loci are restricted to noncoding regions of the DNA to allow for the accumulation of polymorphisms independent of direct selection pressures (Frankham et al. 2004; Abdul-Muneer 2014). However, since they are used in noncoding regions, there are large limitations in using microsatellites to infer information on selection pressures, particularly on individual genes and gene complexes.

There have been several studies describing the characterization of microsatellite loci directly in Asian elephants (Fernando et al. 2001; Kongrit et al. 2008), those developed to complement previously established sets of loci (Lei et al. 2011; Zhang et al. 2015), or those discovered first in African elephants that were found or redesigned to be useful for Asian elephants (Nyakaana and Actander 1998; Eggert et al. 2000; Comstock

et al. 2002; Eggert et al. 2008; Thitaram et al. 2008). These loci differ primarily by the type of microsatellite repeat; Kongrit et al. (2008) characterized dinucleotide loci and Fernando et al. (2001) characterized trinucleotide and tetranucleotide loci. Potential differences in the evolutionary mechanisms and mutation rates of these types of repeats may lead to differing numbers of polymorphisms and corresponding estimates of diversity (Vidya and Sukumar 2005; Goossens et al. 2016) making direct comparisons between studies difficult.

PREVIOUS STUDIES USING CONSERVATION GENETICS IN ASIAN ELEPHANTS

The Asian elephant distribution spans 13 countries in south, southeast, and insular Asia; India, Sri Lanka, Nepal, Bhutan, Bangladesh, Thailand, Myanmar, Lao PDR (hereafter Laos), Cambodia, Vietnam, China, Malaysia, and Indonesia. South Asian populations are primarily found in India and Sri Lanka with only small populations found in Bangladesh, Nepal, and Bhutan (Fernando and Pastorini 2011). While nearly 50-60% of Asian elephants are believed to live within the Indian subcontinent, smaller populations are also scattered throughout southeast Asia (Choudhury et al. 2008; Fernando and Pastorini 2011; Ahlering et al. 2011) with separate subspecies occupying the islands (Fernando et al. 2003). Range wide studies have been largely restricted to inferences based solely on mtDNA (Fernando et al. 2000; Fleisher et al. 2001; Vidya et al. 2009), but local population studies have utilized microsatellites to evaluate nuclear genetic diversity and differentiation. Studies generally determine estimates of nuclear

diversity using expected heterozygosity (H_E) and allelic richness (A_R) and determine mtDNA diversity using nucleotide diversity (π) and haplotype diversity (h).

India – India contains the largest populations of Asian elephants (Fernando and Pastorini 2011); especially in the southern populations of Nilgiris, Anamalai, and Periyar. In these southern populations, Vidya et al. (2005a) determined that despite having the largest number of individuals, they recorded low mtDNA and microsatellite diversity. The largest known Asian elephant population, Nilgiris, was characterized by a single mtDNA haplotype that has been found in numerous populations across the elephant distribution (Fernando et al. 2000; Vidya et al. 2009; Eggert and Ruiz-Lopez 2012; Lei et al. 2012). There was also low differentiation within Nilgiris, but the population was genetically distinct from the other large populations of Anamalai and Periyar. Vidya et al. (2005b) further examined populations across India and found regional genetic differentiation between the country's north, northeastern, central and southern populations. To their surprise, when evaluated overall, the country's genetic diversity was lower than much smaller populations found elsewhere. This low diversity is also seen in range wide studies, where Fernando et al. (2000) found no mtDNA diversity in the small number of samples they examined ($n = 6$), and Vidya et al. (2009) reported low levels when evaluating by region. At smaller scales, Vidya and Sukumar (2005) found social organization of the southern populations was structured by highly related female herds while unrelated males exhibited non-random dispersal throughout the area. Chakraborty et al. (2014) found further evidence of discrete local family groups and evidence against cohesive regional herds of females in Alur area of the Nilgiris population. Based on their

results, they advised against translocation of conflict elephants as it could disrupt local family group and herd dynamics.

Sri Lanka - The Sri Lankan elephant is a currently recognized subspecies; however, genetic studies have argued that it may not warrant subspecies designation. Morphological distinctions describe *E. m. maximus* as the largest and darkest of the Asian elephant subspecies with an unusually large proportion (98% in one population of South Sri Lanka; De Silva et al. 2011) of males missing tusks (Kurt et al. 1995). However, studies in African and Asian elephants have demonstrated that the proportion of tuskless elephants in a population is strongly correlated with poaching pressure (Kurt et al. 1995; Raubenheimer and Miniggiro 2016). While weakly supported by early studies using allozyme loci (Nozawa and Shotake 1990), no further genetic studies have detected a clear distinction between Sri Lankan and Indian elephants (Hartl et al. 1995; Hartl et al. 1996; Fernando et al. 2000; Fleisher et al. 2001). Although Fernando et al. (2000) suggested subspecies designation may not be warranted, they found 10 mtDNA haplotypes that, at the time of their publication, were unique to Sri Lanka and the island was characterized by the highest level of diversity in their range wide study. In a separate range wide study, Vidya et al. (2009) also reported the highest mtDNA diversity was housed in Sri Lanka.

Within Sri Lanka's Ruhuna National Park population, Fernando and Lande (2000) found low-level social structuring in related female groups united by shared mtDNA haplotypes. However, the higher order social structure and/or relatedness organization at a larger scale that has been described in African elephants was not found (Fernando and Lande 2000).

Nepal – One of the largest elephant populations in Nepal is found in Bardia National Park; however, it was functionally extinct from 1970-1980 (Dinerstein 1980). After a large-scale project between India and Nepal established habitat protections and dispersal corridors in 2001, elephants were allowed to naturally recolonize Bardia. Flagstad et al. (2012) evaluated the established population using capture-mark-recapture (CMR) analyses. They determined the population size to be 57 individuals (95% CI = [40, 90]). They found a male-biased sex ratio believed to result from subadult males driving the initial immigration to colonize the unoccupied habitat; however, groups of related females indicate family groups have since immigrated as well. They further determined that recent local recruitment was occurring, as they identified calves in their study. Despite the small population size, genetic diversity was relatively high with no evidence of inbreeding.

Bhutan – Bhutan has been largely understudied using conservation genetic methods. While included in some range wide studies (Fernando et al. 2000; Vidya et al. 2009), I was unable to find a study solely on populations found within Bhutan. However, in their range wide study, Fernando et al. (2000) found that elephants from Bhutan and India were not significantly different, but had moderate levels of diversity when compared to other countries in the study.

Bangladesh – Estimates of Bangladesh's elephant population through dung surveys, interviews, and sightings indicate that there may be 300-350 wild elephants remaining in the country, however 100-150 are believed to have transboundary ranges (IUCN 2004; Islam et al. 2011). To my knowledge, no study has evaluated Bangladesh's

elephants using genetic techniques, and they are notably absent from range wide studies (Vidya et al. 2009).

Thailand – The largest populations in southeast Asia and some of the highest reported levels of genetic diversity in Asian elephants are found in Thailand. When assessing captive individuals with known wild origins, Thitaram et al. (2010) found low genetic differentiation between elephants from different regions of the country, likely due to long standing human traditions promoting translocations. They also reported moderate levels of genetic diversity contained within the captive populations. Reintroduced individuals in Sublanka and Doi Phamuand wildlife sanctuaries were examined by Thitaram et al. (2015) prior to and after their release. They reported high levels of genetic diversity and low relatedness between association groups with many elephants preferentially choosing isolation. These results were likely reflective of the diverse sources from which the reintroduced elephants were obtained. High genetic diversity was also reported by Thongchai et al. (2011) when they evaluated mtDNA in wild elephants from Phuwua, Phukhiao, Phuluang, and Dongyai wildlife sanctuaries.

Myanmar – Myanmar is home to the world's largest semi-captive populations with “captive” individuals released at night or during the off season from the timber and tourist industry, leading to associating and breeding with local wild herds (Lair 1997; Leimgruber et al. 2008; Leimgruber et al. 2011). In a study of semi-captive elephants, Kusza et al. (2018) reported moderate levels of nuclear diversity, but high levels of mtDNA diversity. Overall, their results supported the occurrence of a single regional population with high levels of gene flow. In Vidya et al. (2009), Myanmar had very high levels of mtDNA diversity; including haplotypes from both evolutionary clades. Vidya et

al. (2009) also determined that Myanmar was likely a zone of contact for unrelated β haplotypes following Pleistocene expansion from more southern refugia, which would give rise to high modern-day diversity.

Lao PDR – The elephants of central Laos, specifically those of the Nakai plateau within the Nakai-Nam Theun National Park, were believed to represent the largest elephant population in the country (Duckworth et al. 1999). The Nakai plateau population was previously studied using two different survey methods simultaneously: dung count surveying and genetic capture-mark-recapture (CMR). While dung count surveys produced a population size estimate of 141 individuals (95% CI = [95, 208]), genetic CMR estimated 132 individuals (95% CI = [120, 149]) with greater precision (PCCL 21.97% vs. 80.14%; Hedges et al. 2013). For this population, genetic methods also provided demographic data such as sex, reported high levels of genetic diversity, and demonstrated matriarchal family relationships within herds (Ahlering et al. 2011).

The Sepon Mine population in the Savannakhet district of Laos was estimated at 47 (95% CI = [38, 71]) individuals during a genetic survey (Eggert and Ruiz-Lopez 2011). The levels of genetic diversity reported were lower than that of the Nakai population, but higher than that of populations in India, Sri Lanka, and Vietnam, supporting the conservation value of the elephant populations of Laos (Eggert and Ruiz-Lopez 2011).

An evaluation of the Nam Kading protected area using mtDNA was conducted to evaluate its connectivity to other Laos populations; Nakai and Sepon (Eggert and Budd 2017). The study detected nine mtDNA haplotypes in Nam Kading with only two previously detected in Nakai. These shared mitochondrial haplotypes indicated a historic

maternal relationship between these populations, but low contemporary maternal geneflow. The presence of seven unique haplotypes supported previous conclusions regarding the high levels of diversity found in Laotian elephant populations.

Cambodia – The elephants of the Seima Biodiversity Conservation Area, Cambodia, are thought to be part of one of the more important remaining elephant populations in Indochina (Pollard et al. 2008; Eggert and Ruiz-Lopez 2012). A genetic CMR study estimated the population size at 116 individuals (95% CI = [101, 139]; Eggert and Ruiz-Lopez 2012). This was a rather surprising result considering other methods had estimated the population size at only 30 to 50 individuals. Also surprising was the high level of genetic diversity reported, which was higher than levels detected in much larger populations in India and Sri Lanka (Pollard et al. 2008). In the Phnom Princh wildlife sanctuary in the Eastern Plains of Cambodia, Gray et al. (2014) also reported relatively high levels of genetic diversity in an estimated elephant population size at 136 (SE = 35).

Vietnam – Vietnam's population of elephants is small, with fewer than 100 individuals believed to remain in the wild (Vidya et al. 2007; Ly 2011). In Cat Tien National Park, Vidya et al. (2007) found high levels of mtDNA diversity, but low nuclear diversity and evidence of a recent population bottleneck. They also found that social groups were made up of remnants of different groups of matrilineal lines converged together, likely as a response to disturbance. In their range wide study, Fernando et al. (2000) determined that there was low subdivision between Vietnam and Laos, however, even with a small sample size ($n = 4$), Vietnam had high mtDNA diversity. These high levels of diversity were also reflected in Vidya et al. (2009).

China – Chinese elephant populations have declined rapidly in recent history due to habitat loss and poaching, with the last populations residing along the Myanmar and Laos borders (Zhang 2011). In these remaining populations, Zhang et al. (2015) reported low genetic diversity and genetic CMR determined the smallest population, Nangunhe, consisted of only 28 individuals (95% CI = [24, 32]) while the largest, Xishunagbanna, contained 94 individuals (95% CI = [87, 116]). Overall, Zhang et al. (2015), estimated the total population size of elephants in China at 186 individuals (95% CI = [178, 192]). He et al. (2020) determined that these small populations are isolated with low genetic diversity, and therefore, may soon suffer from inbreeding depression. Their population viability analyses indicated that the construction of corridors and expansion of protected habitat could be necessary to alleviate the potential for inbreeding depression and avoid further declines of China's elephant populations.

Malaysia – There are large differences between the population genetics of elephants found in peninsular Malaysia and those found on the island of Borneo. Those on peninsular Malaysia belong to the Indian elephant subspecies, *E. m. indicus*, while those on Borneo are the proposed subspecies *E. m. borneensis*. While *E. m. borneensis* has been described as having a smaller body size, longer ears and tail length and a lighter skin pigment, Othman et al. (2008) was unable to find a distinctive correlation for these characters when comparing Bornean to peninsular Malaysian elephants. From peninsular Malaysia, mtDNA was examined from multiple populations and the results indicated low genetic diversity overall and at local scales (Elliza et al. 2015). The majority of mtDNA haplotypes produced in the study were shared among populations within the country, but few have been found in populations elsewhere. They determined high gene flow between

Malaysian states, consistent with low differentiation between states, but attributed this to a large number of translocations performed by humans rather than natural elephant movement (Elliza et al. 2015).

Using microsatellite loci and mtDNA sequences, Fernando et al. (2003) found considerable differentiation between Bornean elephants and mainland elephants. The analysis using mtDNA showed that the entire Bornean population was characterized by a single unique and highly differentiated haplotype. Their results supported the hypothesis that Bornean elephants were indigenous to the island and had been isolated since the Pleistocene, contrary to beliefs that the elephants had been introduced during the 16th-18th century. Based on their results, they supported recognizing the Bornean elephant as a separate evolutionarily significant unit so that the proposed subspecies would not be crossbred with other elephants in captivity. Extremely low genetic diversity in Borneo's elephants was also reported by Goossens et al. (2016), who, despite sampling the entirety of their range in Borneo, still only found the single unique mtDNA haplotype. They found low, but significant population structure over a small geographic distance, but were unable to identify potential landscape barriers. While genetic diversity was low, Goossens et al. (2016) warned of the dangers of outbreeding depression that may be experienced if mainland elephants were translocated in an attempt augment the population size and diversity of Bornean populations.

Indonesia – Elephants in Indonesia are found solely on Sumatra as elephants on Borneo are restricted to the Malaysian region. Sumatran elephants are also another unique subspecies, *E. m. sumatranus*. Sulandari and Zein (2013) found mtDNA haplotypes unique to the Sumatran island, but mtDNA diversity overall was very low.

Moßbrucker et al. (2015) determined the largest populations in central Sumatra are fragmented into two isolated subpopulations. They determined population size in the first, Sumai, as 99 individuals (95% CI = [86, 125]), and the second, in the RiauJambi area, as 44 individuals (95% CI = [37, 56]). They found strongly female-biased sex ratios and an overall young age structure, both of which suggest the presence of elephant killing for the ivory trade.

PURPOSE OF THIS DISSERTATION

For large animals such as elephants with vast spatial requirements, the highly fragmented patchwork of habitat presents problems for dispersal and overall connectivity of populations. As human development expands, elephants will continue to be displaced, increasing the likelihood of human elephant conflict. Genetic techniques offer unique and effective ways to analyze population structure and size, habitat use, social structure and movement of individuals in populations that would be impossible to capture through more traditional methods. Sampling using non-invasive techniques (i.e., collection of feces) not only maximizes the number of individuals that can be included in a study, but also limits the potentially life-threatening physiological stresses that come with blood and tissue collection.

These non-invasive genetic techniques are used in Chapter 2 to examine the effects of direct conflict on the population structure and demography of Myanmar's elephants. The population of elephants in Bago Yoma, Myanmar, have experienced repeated human-elephant conflict both at a widescale level with poaching for the developing skin trade and more locally with elephants participating in agricultural crop-

raiding. Non-invasive samples from the current population were used to better understand the overall population structure and demography of these heavily impacted elephants as well as to identify characteristics of known crop-raiders.

Chapter 3 evaluates the effects of habitat transformation and disturbance of the elephants of the Nakai Plateau, Laos, following the construction of the Nam Theun 2 hydroelectric dam and subsequent inundation of the Nakai Plateau in central Laos. As described previously, the Nakai population was first surveyed in 2006 prior to the construction of the hydroelectric dam. The population's combination of high genetic diversity and largely intact social structure identified it as of high conservation value despite its relatively small size (Ahlering et al. 2011; Hedges et al. 2013). A complementary population survey in 2018/2019 was conducted to determine the effects of the hydroelectric dam on the population structure, diversity, and demography of the remnant elephant population.

In Chapter 4, genetic data are used to identify range wide hotspots of diversity and evolutionary distinctiveness in the Asian elephant. By standardizing diversity data collected using differing numbers and types of microsatellite loci, this study generates a yardstick population and calibration model capable of identifying diversity rich populations across the distribution. I also evaluate the evolutionary distinctiveness of population lineages using their mitochondrial haplotypes and to identify range wide hotspots of genetic diversity for conservation prioritization.

Overall, the purpose of this dissertation is to aid directly in the conservation of the Asian elephant by identifying the impacts of human-elephant conflict, habitat

fragmentation, and genetic hotspots to inform decisions that ensure the future of this iconic endangered species.

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CHAPTER 2

POPULATION STRUCTURE AND DEMOGRAPHY OF MYANMAR'S CONFLICT ELEPHANTS

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ABSTRACT

Despite containing the largest amount of unfragmented landscape in the Asian elephant (*Elephas maximus*) range, Myanmar has high levels of human-elephant conflict. The Bago Yoma mountain region of central Myanmar has previously been identified as an elephant conflict hotspot, characterized by high levels of crop-raiding and illegal killing of elephants for the ivory and skin trades. Non-invasive fecal sampling was used to evaluate the population structure and demography of wild and captive elephants in the Bago Yoma using microsatellite loci and mitochondrial DNA in combination with crop-

raiding status, age, and sex. Teams were able to collect 252 samples from wild elephants –119 directly following conflict events– and 25 from a captive elephant camp. The population was biased toward subadults, which could be an important contributor to the high rates of conflict, as these individuals may lack the experience to avoid dangerous behaviors. Conflict elephants were primarily male, although both sexes and all ages engaged in crop-raiding, including females with juveniles. Elephants that committed solo raids were all male, while larger raiding parties often included both related and unrelated individuals of both sexes. Repeat offenders were common. These wild elephants were characterized by high levels of genetic diversity, differentiated from local captive populations, and are thus valuable for the species' conservation. Overall, the elephants of Bago Yoma, Myanmar have been heavily affected by conflict, and crop-raiding deterrents could utilize the knowledge presented to aid in the preservation of this population.

INTRODUCTION

The Asian elephant (*Elephas maximus*) is a globally recognized endangered species with highly dispersed populations of limited connectivity throughout their habitat and geographic range (Leimgruber et al. 2003; Choudhury et al. 2008). The largest amount of unfragmented habitat in the species' range is found in Myanmar, a country that has been described as a potential stronghold for Asian elephant conservation (Leimgruber et al. 2003; Bhagwat et al. 2017). The people of Myanmar have a long and complex relationship with elephants, with widescale usage in the timber industry leading to the world's largest captive elephant population (Leimgruber et al. 2008). Myanmar's

working elephants are sometimes considered semi-captive, as they are allowed to feed and roam in habitats surrounding their camps at night and during off-seasons, albeit usually constrained by drag chains or hobbles. During these time periods they may interact and even breed with wild individuals (Crawley et al. 2019; Seltsmann et al. 2019).

Myanmar's wild elephant population has declined dramatically since the mid-1900s, and more recent assessments suggest that populations are still declining (Leimgruber et al. 2011). Much of this population decline had previously been attributed to a constant off-take of wild elephants to supplement the country's working elephant population (Leimgruber et al. 2008). However, live capture of elephants for this industry officially ended in the early 2000s following directives from the government, and the continued population declines are more recently due to habitat loss, conflict, and poaching (Leimgruber et al. 2008; Sampson et al. 2018).

The illegal killing of wild elephants for their products is among the greatest threats to the future of Myanmar's elephant populations (Sampson et al. 2018). Recent work on the hunting and local wildlife trade in Myanmar indicated that local amassing of illegal wildlife products, primarily driven by Chinese demand (Nijman and Shepherd 2014), may be much higher than previously thought and contribute significantly to poaching pressures on many local endangered fauna (McEvoy et al. 2019). In addition to ivory, market surveys along the Myanmar-China border have shown an increasing demand for elephant skin, genitalia, and other body parts that are largely sourced from wild elephants in Myanmar (Nooren and Claridge 2001; O'Connell-Rodwell and Parry-Jones 2002; Nijman and Shepherd 2014). A rise in demand for skin products in particular (Nijman and Shepherd 2014; Elephant Family 2019), has resulted in increasing rates of

illegal killing of elephants across Myanmar (Sampson et al. 2018). Between 2015 and 2017, Sampson et al. (2018) identified over 40 poached elephants including seven of their own collared individuals and local reports of dozens more in nearby regions. While tusked males are rarer in this population due to previous poaching selective pressures (Kurt et al. 1995), only a single individual killed was a tusked male. Further, the majority of the carcasses were skinned, confirming the shift in demand from traditional ivory products to skin, and posing a much greater risk to the elephant populations as now females may also be targeted.

Remnant elephant populations in Myanmar inhabit forest fragments alongside the over 60 million people that also reside within the country (Leimgruber et al. 2011; Songer et al. 2016). A systematic assessment using dung counts along transects suggested that there were about 2,583 elephants in the entirety of the Bago Yoma, the mountainous region near the former capitol and largest city, Yangon, in 1998 with approximately 56 elephants in the Okkan Forest Reserve of the southern foothills area (Varma et al. 2008). People have converted large tracts of forest into agriculture lands, primarily rice paddy, in the foothills of the Bago Yoma since the 1990s. This patchwork of forest habitat alongside croplands entices elephants to exploit the high nutritional value of crops at the expense of local farmers (Webber et al. 2011). Elephant crop-raiding can be disastrous to farmers, as the animals not only consume valuable agricultural products, but also cause large-scale collateral damage to crops, houses, farming equipment (Sampson et al. 2019), and even livestock (Rodriguez and Sampson 2019).

The compression of local elephant populations alongside expanding small-scale agriculture have led to human-elephant conflict (HEC). The southern foothills of the

Bago Yoma have likely seen the most significant increases in conflict of any area in Myanmar since the 1990s. Research on human-wildlife conflict has demonstrated that continued local conflict (*e.g.*, crop-raiding, property damage) can discourage local conservation practices, increase tolerance of illegal poaching, and decrease overall support for conservation efforts (Fenio 2014; Kansky et al. 2016). In this study, we sought to understand the dynamics of HEC, specifically the demography and population structure of a conflict population. We were interested in assessing the elephant population in the HEC area and determining more about elephants in the population that are involved in conflict, including whether some individuals are more likely to engage in conflict based on previous conflict history, relationship, sex, and age group. These experimental goals would contribute directly to Asian elephant conservation and management by informing appropriate conflict mitigation strategies based on demography and population structure.

METHODOLOGY

Study Area – Our sampling sites were located in the southern foothills of the Bago Yoma mountain range in central Myanmar, approximately 60 km north of Yangon, near the Tak Kyi and Tharrawaddy townships (Figure 2.1). Elephant habitat consists of disturbed mixed deciduous forests with semi-evergreen forests alongside developed agriculture fields. Agriculture in this area consists mostly of small-scale operations of rice and sugarcane. The area also contains a highly profitable stretch of teak forest that has been subject to substantial logging operations, using local captive elephant camps, for the past 130 years (Salter 1983). The major elephant populations of the region inhabit the

nearby forest reserves of South Zamari, North Zamari, Yenwe, Idokan and Okkan (Varma et al. 2008). The elephant population of the southern foothills is likely to be connected to the nearby Okkan Forest Reserve.

Sample Collection – In fall 2015 and spring 2016, local villagers and staff from the Ministry of Natural Resources and Environmental Conservation (MONREC) were trained to monitor elephant activity, complete standardized reports, collect, measure, and preserve fecal samples, and record GPS coordinates (when devices were available) after conflict events such as crop-raiding or destruction of property. The sampling teams also collected samples from sites such as within the forest or along roads using two methods: 1) opportunistic sampling and 2) transect sampling. In the opportunistic sampling method, team members gathered fecal samples when they encountered a dung pile by chance or in the course of other research-related activities. In the transect method, the sampling teams traveled all roads within the study area searching for signs of elephant crossings, easily identifiable by their fresh tracks. The teams would then track the elephant(s) until they found dung or could not follow the trail any further. Two rounds of the transect method were completed within the study period, each lasting approximately two weeks. Samples were also collected from captive elephants at the local government-owned timber camp, Myaing Hay Wun elephant camp, where working elephants are released every evening and recaptured every morning by MONREC staff.

The age of individual elephants was estimated from the average circumference of up to three dung boli, following criteria described in Tyson et al. (2002). For genetic analyses, teams collected 10 mg of fresh elephant dung (< 24 hours old) preserved in Queens College buffer (20% DMSO, 0.25 M EDTA, 100 mM Tris, pH 7.5, saturated

with NaCl; Amos *et al.* 1992). All samples were boiled at 70° C for 30 minutes in compliance with USDA-APHIS import requirements and stored at ambient temperatures until exported to the United States for analysis.

Genetic Identification – We conducted DNA extraction using a modified QIAamp DNA stool Mini Kit (Qiagen, Germantown, MD) protocol optimized for use with elephant dung (Archie *et al.* 2008). We further optimized this protocol by centrifuging 2000 µl of the raw sample at 12000 RPM for 1 min, discarding the liquid buffer, adding raw sample to the same tube to a volume of 2000 µl and centrifuging again until ~ 800 µl of concentrated dung sample was collected. Samples were incubated a minimum of 24 hr at 56° C in a total volume of 2000 µl containing 20 mg Proteinase K and Stool Lysis Buffer (Buffer ATL, Qiagen) before extraction. We also extended final DNA elution time to 30 min for maximum recovery of DNA. For samples with poor DNA concentrations, we conducted an additional extraction and concentrated elution using an ethanol precipitation. We amplified DNA using eight microsatellite loci previously characterized in Asian elephants (Kongrit *et al.* 2008, Table 2.1) in a multiplex polymerase chain reaction (PCR) using Platinum Master Mix and GC Enhancer (Applied Biosystems), 2 mM fluorescently tagged primer mix, and 0.8 mM BSA. All reactions included positive controls to standardize allele scoring and negative controls to detect contamination of PCR reagents. The PCR profile consisted of an initial incubation at 95° C for 2 min; 40 cycles of 94° C for 30 sec, annealing at 58° C for 90 sec, 72° C for 60 sec, followed by a final extension cycle at 60° C for 30 min. Amplification products were verified in a 2.5% agarose gel stained with GelStar (Lonza).

Amplified products were genotyped using an ABI 3730xl DNA analyzer (Thermo Fisher Scientific, Waltham, MA) at the University of Missouri DNACore facility with added 600 LIZ size standard. Samples were repeated to detect genotyping errors and to ensure genotyping consistency across reactions. All genotypes were scored in GeneMarker v. 1.9.7 (Holland and Parson 2010). We tested for allelic dropout, scoring error due to stuttering, and null alleles in Microchecker (Van Oosterhout et al. 2004). We calculated the probability of identity for randomly chosen individuals (PI_{random}) and for siblings (PI_{sibs}) in GenAlEx v. 6.41 (Peakall and Smouse 2012). Unique individuals were identified using the thresholds of Waits et al. (2001) with fuzzy matching at up to two loci considered during identity analysis in Cervus (Kalinowski et al. 2007) in combination with fecal bolus circumference.

We determined the sex of each individual by amplifying sex specific fragments - two short Y-specific fragments (SRY1 and AMELY2) and a longer X-specific fragment (PLP1) and visually inspecting the amplified products on a 3% agarose gel (Ahlering et al. 2011a). Each sexing PCR and gel verification was repeated at least three times for confirmation to avoid misidentification due to allelic drop out.

We randomly selected a subset of identified individuals ($n = 64$ wild, 7 captive) and sequenced a 593 bp mitochondrial DNA (mtDNA) fragment spanning the C terminal of cytochrome b, threonine and proline tRNAs, and part of the noncoding mitochondrial control region using primers developed specifically for the Asian elephant (Fernando et al. 2000). PCR reactions were conducted in 25 μ l volumes comprised of 0.4 μ M primers (forward and reverse), 1X PCR Gold Buffer (50 mM KCl, 8 mM Tris-HCl), 0.2 mM dNTPs, 2.0 mM MgCl₂, 0.8 mM BSA, and 0.5 U Amplitaq Gold DNA Polymerase

(Thermo Fisher Scientific, Waltham, MA, USA). The PCR profile was: 95° C for 10 min; 45 cycles of 95° C for 1 min, 55° C for 1 min, 72° C for 1 min, followed by a final extension at 72° C for 10 min. Amplification products were verified in a 2.5% agarose gel stained with GelStar (Lonza), purified using ExoSap-IT (USB), and sequenced bidirectionally in an ABI 3730xl DNA analyzer (Thermo Fisher Scientific, Waltham, MA) at the University of Missouri DNACore Facility. The resulting chromatographs were aligned using ClustalW and manually trimmed in Geneious v. 8.0.5 (Kearse et al. 2012). Sequences that differed by at least one nucleotide were collapsed into haplotypes using FaBox v. 1.41 (Villesen 2007) and compared to previous studies directly from the literature or to the NCBI GenBank database (Benson et al. 2007) using the Basic Local Alignment Search Tool (BLAST).

Population Analyses – Population density plots were generated for each sex based on the distribution of bolus circumference (as a proxy for age; Tyson et al. 2002) in RStudio v. 3.5.2 (R Core Team 2018). Age distributions were tested for normality using a Shapiro-Wilks test for normality and tested for differences between the sexes using a Mann-Whitney test in R (R Core Team 2018).

We tested for deviations from expectations under Hardy-Weinberg equilibrium (HWE) and for linkage disequilibrium (LD) in GenePop (Raymond and Rousset 1995), with a standard Bonferroni correction for multiple comparisons for all individuals and for the sexes separately. We calculated observed heterozygosity (H_O), and expected heterozygosity (H_E) in GenAlEx v. 6.41 (Peakall and Smouse 2012) and rarefied allelic richness (A_R) and private allelic (A_P) were generated using HP-Rare v. 1.1 (Kalinowski 2005) for the population as a whole as well as independently for each sex and age class.

Per locus H_E and H_O for each were arcsine square root transformed and A_R and A_P were fit to gamma distributions for statistical analysis in RStudio v. 3.5.2 (R Core Team 2018). Significant differences between sexes and age classes were determined by applying linear models with locus as a fixed effect using the LME4 package v. 1.1-23 (Bates et al. 2015) followed by an ANOVA in the CAR package v. 3.0-9 (Fox and Weisberg 2019). Post-hoc testing was completed by applying a Tukey test in Multcomp v. 1.4-13 (Hothorn et al. 2008). We calculated effective population size using NeEstimator v. 2.1 (Do et al. 2014) with a minimum allele frequency cutoff at 0.020. For mtDNA, I calculated haplotypic diversity (h), and nucleotide diversity (π) and compared differences between sexes using *genetic_diversity_diffs.R* v. 1.0.6 (Alexander et al. 2016) with 10 000 permutations.

We tested for population structure using bayesian model-based clustering in Structure v. 2.3.4 (Pritchard et al. 2000). We applied an admixture model with no priors and tested up to 10 genetic clusters ($K = 1-10$) with 10 replicates for each K using 50 000 burn-in steps and 250 000 MCMC replicates. We determined the number of genetic clusters based on the probability of K ($\ln \{Pr(X|K)\}$). We evaluated nuclear differentiation (F_{ST}) between sexes, age classes, and captive individuals in GenAlEx v. 6.41 (Peakall and Smouse 2012) using analysis of molecular variance (AMOVA). We also evaluated the number of migrants entering the population per generation (N_m) between captive and wild elephants by sex using 9 999 permutations in GenAlEx v. 6.41 (Peakall and Smouse 2012). We tested for mtDNA differentiation (ϕ_{ST}) between sexes and with the captive population in Arlequin, assessing the significance of differentiation using the empirical p-

value generated from 9 999 permutations (Excoffier et al. 2005). We applied a standard Bonferroni correction to determine statistical significance (Neyman and Pearson 1928).

For samples collected during conflict events, we evaluated the demographic composition of raiding parties. We estimated first order pairwise relationships (parent-offspring, full sibling, half sibling or unrelated) between elephants in a raiding party in ML-Relate (Kalinowski et al. 2006). We further evaluated shared characteristics such as genetic identity, sex, and age between elephants found at multiple conflict events, deemed “repeat offenders”.

RESULTS

Collection and Individual Identification – Local villagers, MONREC staff, and sampling teams collected 252 Asian elephant dung samples from the wild elephants in the southern Bago Yoma foothills; of these, 119 were collected following conflict events. We determined 6 loci were necessary to distinguish individuals and siblings with high confidence ($PI_{\text{random}} = 5.84 \times 10^{-6}$; $PI_{\text{sibs}} = 5.58 \times 10^{-3}$). Therefore, 25 samples were excluded from further analysis for failing to reach this threshold. Within the remaining 227 samples, we identified 127 unique individuals with 100 recapture events based on a combination of genotype and bolus circumference measurements. Within and surrounding the captive elephant camp, teams collected an additional 25 samples found to represent 21 of the captive elephants. A single sample believed to be a wild individual was determined to be a recapture of a captive individual. Recapture events of wild elephants consisted of 38 individuals with up to 7 recaptures of the same individual.

Demography – Of the 127 identified wild individuals, 82 (64.6%) were male while 45 (35.4%) were female. We found 17 juveniles (fecal bolus circumference < 30 cm; 11M, 7F), 57 subadults (30 – 42 cm; 34M, 22F), and 30 adults (> 42 cm; 21M, 9F), while 23 were of unknown age (16M, 7F) due to the lack of intact fecal boli. Females in particular were heavily subadult biased, but males also were skewed toward a subadult dominant population. Fecal bolus circumferences were normally distributed ($W = 0.979$, $p = 0.098$), and distributions were not significantly different between sexes (two-tailed; $W = 1200.5$, $p = 0.721$; Figure 2.2).

Population Status – We determined 3 of the 8 loci did not conform to expectations under Hardy-Weinberg Equilibrium (HWE; EMU02, EMU09, EMU12), but no loci showed evidence of linkage disequilibrium. However, when sexes were tested separately, females had no loci out of HWE while males were out of HWE again for three loci. We found a mean $H_O = 0.573 \pm 0.049$ SE and $H_E = 0.676 \pm 0.031$ SE for all individuals (Table 2.1). The mean number of alleles overall per locus was 4.875 ± 0.350 SE. Sexes and ages were not significantly different on any diversity metrics. We estimated the effective population size at 60.0 (95% CI = [42.2, 89.9]) with a harmonic mean of 116.1.

Using mtDNA, we identified four previously discovered haplotypes BO (AY245808), BH (AY245803), BQ (AY245816), and BL (AY245804; Fernando et al. 2003). BH, BL, and BQ have previously been found in Myanmar among other countries, whereas BO has previously been found in Vietnam and Sri Lanka (Vidya et al. 2009). In the wild elephant population, we found BH and BO in high frequencies (51.6% and 45.3% respectively), while BQ and BL were rare (1.5% each). For the wild elephants,

haplotype diversity was 0.537 ± 0.024 SE and nucleotide diversity was 0.004 ± 0.002 SE. Using wild males only, haplotype diversity was 0.544 ± 0.036 SE with a nucleotide diversity of 0.003 ± 0.002 SE, while for wild females, haplotype diversity was 0.634 ± 0.069 with a nucleotide diversity of 0.004 ± 0.003 SE (Table 2.1). In the captive population, we only identified BQ and BH; although the frequency of BQ was much higher (57.1%). Haplotype diversity was 0.571 ± 0.120 SE while nucleotide diversity was 0.006 ± 0.004 SE in the captive elephants. Haplotype diversity and nucleotide diversity were not significantly different between wild males and wild females, or between wild and captive individuals.

Structure and Differentiation – We found no major population structure breaks ($K = 1$) among wild individuals, however when the captive population was included, we found minor population structure ($K = 3$, mean ($LnProb$) = -2942.90), breaking particularly from wild females (Figure 2.3).

Nuclear genetic differentiation (F_{ST}) was significant between the captive population and wild males ($F_{ST} = 0.032$, $p = 0.000$) and wild females ($F_{ST} = 0.050$, $p = 0.000$), but not between wild males and wild females following Bonferroni correction. The number of migrants per generation (N_m) between wild males and captives was 7.538 while between wild females and captives was 4.73, both substantially lower than the 50.635 between wild males and wild females. There were no significant differences between the age distributions by sex or wild and captive status, following Bonferroni correction. Using mtDNA, both wild males ($\phi_{ST} = 0.481$, $p = 0.000$) and wild females ($\phi_{ST} = 0.372$, $p = 0.009$) were significantly differentiated from captives, although not from each other.

Conflict Elephants – Samples were collected immediately following 32 conflict events consisting of crop-raiding or destruction of property. At 47% (n = 15) of the conflict events, we detected DNA from a single elephant, and we therefore assumed these raids were committed by lone individuals. As elephants have a mean defecation rate of 18 times per day, it was unlikely that an individual present during a conflict event did not defecate at least once during that time. Furthermore, all of these solo raids were committed by males, consisting of 33% subadults and 66% adults. In conflict events with more than one elephant present, deemed ‘raiding parties’, we detected 4.50 ± 0.76 SE elephants in a party on average. The majority of these parties (56%) were of mixed relationships: typically, a core group of highly related individuals (parent-offspring and/or full siblings) accompanied by 1-2 unrelated individuals. In raiding parties, 69% had at least one subadult or adult female present and 50% had at least one juvenile. Only one raiding party consisted entirely of females, which were unrelated. Parties consisting entirely of unrelated individuals made up 33% of raiding parties and were almost exclusively males (all but the one mentioned previously). We found 15 repeat offenders, elephants present at more than one conflict event, that were 80% male and 66% subadults. The greatest repeat offender was a subadult male located at five separate conflict events; both as an individual raider and with raiding parties. In all 32 conflict events, at least one repeat offender was present in 72% of raids.

DISCUSSION

This study is the first systematic assessment of a wild elephant population in Myanmar based on genetic analysis. At an expert workshop in 2002, Burmese elephant

experts estimated the population for the entirety of the Bago Yoma to be between 200-240 (Leimgruber et al. 2011). The 127 wild elephants identified in this small region of the Bago Yoma foothills could indicate that a large proportion of the population is concentrated there, that elephants across a broader range visit the area frequently, or that overall expert estimates for these areas are too low or outdated.

The Bago Yoma population seems to show higher population densities than were previously found or are currently estimated to be in the region's major elephant conservation areas. Subadult males are the primary dispersers in Asian elephants (Sukumar 1991), which could account for their high proportion in the Bago Yoma. The recent surge of small-scale agriculture into the Bago Yoma foothills may be enticing the nearby elephant populations, including those in the Okkan Forest Reserve, to take advantage of the highly accessible and nutritious resource, leading to an overall increase in the local population density. This high population density could also be driven by high levels of poaching in the major conservation areas as elephants seek refugial habitat; however, these conservation areas are generally protected by their overall inaccessibility (Varma et al. 2008). Regardless, this highly dense local population may be temporary or harmful, as the landscape changes may create an ecological trap (Hale and Swearer 2016) in which elephants attracted to the high nutrient content in crops are placed at increased risk of poaching because of their increased accessibility and crop-raiding.

In the southern foothills of the Bago Yoma, we found that all sexes and ages participated in crop-raiding, although the majority of raids were committed by males, as seen in other populations (Sukumar 1990; Williams et al. 2001; Ekanayaka et al. 2011). Males were found raiding alone or in raiding parties, while females raided only in parties.

Raiding parties themselves appear to resemble the female relative-based herd structure seen elsewhere in elephants (Vidya and Sukumar 2005), except that they generally included 1-2 unrelated individuals. We were unable to determine if all elephants in a raiding party were continuously together in the general population or if the related herd engaging in conflict was drawing in unrelated individuals to participate. Elephants will preferentially select habitat by considering resource availability in comparison to disturbance or risk and unlike males, female herds will typically sacrifice a high-resource for decreased risk, particularly when juveniles are present (Srinivasaiah et al. 2012). However, we found that juveniles were present in 50% of raiding parties. The young age structure and subadult dominance in herds could be a factor in this finding, as experienced matriarchs would be expected to protect young, inexperienced mothers and juveniles by avoiding such high-risk activity (McComb et al. 2011). We also found that repeat offenders were extremely common and present in the majority of raids. Repeated conflict can be detrimental to their health as elephants that engage in conflict events spend less time resting and feeding, have increased overall stress, and long-term conflict can lead to deterioration of herd structure (Srinivasaiah et al. 2012).

The skewed age distribution, specifically the low frequency of adults, may also be a consequence of the region's high levels of poaching. While elephant poaching is typically associated with the ivory carried by males, more recent poaching patterns observed in Myanmar seem to target males and females indiscriminately for the trade in meat and skin (Sampson et al. 2018) as the majority of males no longer possess tusks due to previous poaching pressure (Kurt et al. 1995; Elephant Family 2019). This is supported by the discovery of carcasses from 20 elephants of both sexes found in a single site in the

Ayeyarwady delta region (Sampson et al. 2018). The loss of females would be particularly detrimental to conservation due to their long gestation period, long inter-calving period, long-time to sexual maturity, and low reproductive rate (De Silva and Leimgruber 2019). It should be noted however, that since sampling efforts were concentrated around conflict events, additional adult females may have been present but undetected, although this would mean that the overall density was even higher in the region. Regardless, the high population density in this area could increase poaching of large elephants that would otherwise be better protected in the inaccessibility of the forest reserves.

The value of protecting this population of elephants from threats such as poaching is also evident in its high levels of genetic diversity, valuable for the species conservation. The high levels of genetic diversity ($H_E = 0.676$) detected in Bago Yoma is comparable to other significant populations in southeast Asia such as those in Cambodia (Pollard et al. 2008), Thailand (Thitaram et al. 2010), Laos (Ahlering et al. 2011b), and is higher than populations found in China (Zhang et al. 2015), Sumatra (Moßbrucker et al. 2015), Borneo (Goossens et al. 2016), and India (Vidya et al. 2005). Despite having a high level of nuclear diversity, mtDNA diversity was relatively low ($h = 0.537$; $\pi = 0.004$). We found lower haplotype diversity than previous studies in Myanmar's captive populations (Kusza et al. 2018), Laos (Ahlering et al. 2011b), Thailand (Fickel et al. 2007) and Sri Lanka (Vidya et al. 2009). In studies of African elephants, high nuclear diversity in combination with low mtDNA diversity was associated with a male gene flow bias (Nyakaana and Arctander, 1999), indicating an unequal contribution of diversity by the sexes. The males of this population also had loci out of Hardy-Weinberg

equilibrium whereas the females did not. This phenomenon can occur upon the mixing of individuals from separate populations and as the dispersing sex, this can signify outside males joining this population, driving the high levels of diversity found here.

Captive elephants in Myanmar are often believed to have high levels of genetic exchange with wild elephants, with calves born to captive cows believed to be sired by wild bulls (Kusza et al. 2018). However, this phenomenon could be happening less than previously believed, as we found significant genetic differentiation between captive and wild individuals using nuclear and mtDNA methods with low levels of generational migrants detected. The results of population structure analysis indicated that the captive population was more similar to wild males than wild females, lending support to possible low levels of unisexual genetic exchange, but overall separation was evident. While this genetic difference is likely not large enough to result in outbreeding depression as is of concern in more diverged populations (Goossens et al. 2016), conservation efforts should not rely solely on the captive population for the preservation of elephant diversity in Myanmar. Despite being the largest captive population of Asian elephants in the world, it is unlikely to preserve the same diversity found in the wild populations and may not adequately maintain the valuable Myanmar genetic landscape for long-term conservation of the species.

Overall, the Bago Yoma is characterized as a high HEC area (Leimgruber et al. 2011), with encroaching towns and villages engaged in conflict with elephant behaviors such as crop-raiding. The combination of a high elephant population density that exhibits high-risk behavior such as crop-raiding with juveniles, and a low number of adults overall may be compounding the risks of conflict in this area. Understanding the

population structure can help wildlife managers develop more effective and efficient conflict mitigation strategies. Controversial methods to control crop-raiders, such as chili and electric fences (Davies et al. 2011; Gunaryadi et al. 2017) or translocations (Fernando et al. 2012), could also be dangerous for juvenile survival. These methods are typically directed toward males, but less dangerous methods may suffice to deter female herds, particularly those with juveniles, as they are more likely to perceive risk over reward (Sukumar 1991; Ekanayaka et al. 2011; Srinivasaiah et al. 2012). Further studies should therefore also consider demographic and population structure of conflict elephants that may influence the mitigation strategy's success. Despite the high levels of conflict found with this population, the southern foothills region of the Bago Yoma also harbors high levels of genetic diversity valuable for the species conservation that may not be represented in Myanmar's captive populations. Threats such as continued poaching and HEC place valuable elephant populations, such as in the Bago Yoma, at further risk, despite their notable value for the preservation of this iconic endangered species.

ACKNOWLEDGMENTS

We wish to thank the Myanmar Ministry of Natural Resources and Environmental Conservation for their assistance and permission to conduct this research, as well as Aung Myo Chit, Muang Muang Chay, and Dr. Zaw Min Oo for their help with in-country logistics. This project would not have been possible without the mahouts at Myaing Hay Wun elephant camp and the rest of the field crew. A portion of this research was funded through the University of Missouri Trans World Airline Scholarship program and Sigma

Xi Grant-in-aid of research and the NSF Graduate Research Fellowship Program. This study was funded in part by the USFWS Asian Elephant Conservation Fund (#ASE1539).

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TABLES

Table 2.1: The nuclear diversity including observed heterozygosity (H_O), expected heterozygosity (H_E), and Hardy-Weinberg Equilibrium (HWE) status departure indicated with an asterisk (*), rarefied allelic richness (A_R) and private allelic richness (A_P) per microsatellite locus and overall, as well as the mtDNA haplotype diversity (h) and nucleotide diversity (π) for all wild individuals and sexes separately.

Locus	ALL			FEMALES					MALES				
	H_O	H_E	HWE	H_O	H_E	HWE	A_R	A_P	H_O	H_E	HWE	A_R	A_P
EMU01	0.543	0.677		0.478	0.669		3.969	0.098	0.573	0.683		3.899	0.028
EMU02	0.701	0.607	*	0.674	0.637		3.000	0.000	0.720	0.588	*	3.000	0.000
EMU09	0.333	0.592	*	0.378	0.543		4.484	0.108	0.315	0.620	*	4.815	0.439
EMU10	0.584	0.609		0.689	0.627		5.488	0.174	0.531	0.601		5.758	0.443
EMU12	0.410	0.590	*	0.525	0.582		4.906	0.350	0.359	0.594	*	4.640	0.084
EMU13	0.648	0.752		0.578	0.766		4.987	0.191	0.679	0.741		4.806	0.011
EMU14	0.642	0.789		0.605	0.777		5.000	0.000	0.654	0.792		5.000	0.000
EMU17	0.724	0.789		0.825	0.792		5.966	0.126	0.662	0.783		5.870	0.030
MEAN	0.573	0.676		0.594	0.674		4.725	0.131	0.562	0.675		4.724	0.129
SE	0.049	0.031		0.050	0.033		0.325	0.040	0.054	0.031		0.331	0.069
h	0.537 +/- 0.024			0.634 +/- 0.069					0.544 +/- 0.036				
π	0.004 +/- 0.002			0.004 +/- 0.003					0.003 +/- 0.002				

FIGURES

Figure 2.1: Map of the primary study area in the southern foothills of the Bago Yoma in central Myanmar with samples collected indicated as conflict, general collection, or captive.

Figure 2.2: Demographic density of wild elephants as shown by distribution of fecal bolus circumference as a proxy for age for each sex. Size thresholds for juvenile (> 30 cm), subadult (30 cm – 42 cm), and adult (> 42 cm) from Tyson et al. (2002) are shown. Subadult dominant populations for both sexes were normally distributed and did not differ significantly between sexes.

Figure 2.3: Analysis of nuclear microsatellite data structure revealed three genetic clusters ($K = 3$, $(LnProb) = -2942.90$) and support the captive elephants' differentiation from both male and female wild elephants. However, higher genetic similarity, although still low, could be shared between captive elephants and wild males than wild females.

CHAPTER 3

EFFECT OF DISRUPTION ON A HIGH-VALUE ELEPHANT POPULATION AS A RESULT OF A HYDROPOWER PROJECT

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ABSTRACT

Habitat loss and fragmentation are among the leading contributors to the endangered status of Asian elephants (*Elephas maximus*). In 2006, the Nakai plateau contained the largest known elephant population in the Lao People's Democratic Republic (Lao PDR; Laos), and was among the highest genetic diversity reported in Asian elephants. In 2008, the closure of the Nam Theun 2 hydroelectric dam inundated much of the plateau, with substantial elephant habitat lost as a result. We therefore examined the Nakai elephant population using genetic sampling in 2018 and 2019 and compared our microsatellite, mtDNA, and demographic results to a pre-dam-closure

genetic survey in 2006 to help understand the effects of the hydroelectric project. We also collated and reviewed reports on elephant presence, movements, and dispersal, mainly, but not solely relating to human–elephant conflict (HEC), on the Nakai plateau and the wider landscape. Overall, when we compared the 2018/19 elephant population with that prior to inundation of the plateau, we found a decrease in genetic diversity and an altered demographic structure, both of which raise concerns about long-term population viability. In addition, inundation of the plateau resulted in a major increase in HEC locally, at least initially, and the creation of new, serious HEC problems some 100km from the Nakai plateau. Such landscape-level effects are an under-appreciated impact of large hydropower projects. These projects are of economic importance throughout much of Laos and southeast Asia, and this study has important implications for mitigating their impact.

INTRODUCTION

Human development and encroachment can fragment wildlife habitat, block migration routes, and facilitate poaching of wild animals, including elephants (Choudhury 2004). The Asian elephant (*Elephas maximus*) is listed as Endangered in the IUCN Red List of Threatened Species and on CITES Appendix I (Williams et al. 2020). It is threatened by continued habitat loss and fragmentation, poaching, and removal from the wild, both legal and illegal (Sukumar 1989; Leimgruber et al. 2003; Williams et al. 2020). Elephants may remain in an area following recent habitat transformation; however, doing so can place them and nearby human populations at extreme risk of conflict (Kushwaha and Hazarika 2004). In addition, as elephant habitat becomes less

suitable due to decreased area and/or increased fragmentation, it becomes less able to support a viable population over the long term (Leimgruber et al. 2003).

The Lao People's Democratic Republic (Lao PDR, hereafter referred to as Laos) historically contained extensive elephant habitat and travel corridors (Khounboline 2011). While elephant populations have declined, as they have across much of southeast Asia, central Laos, especially the Nakai plateau area (Figure 1), was thought to be occupied by one of the two most important elephant populations in the country (Duckworth and Hedges 1998). More generally, the Nakai–Nam Theun National Park (NNT NP), spanning the north-east half of the Nakai plateau into the Annamite Mountains up to the Lao-Vietnam border, is considered a biodiversity hotspot, and has been recently nominated for inclusion in the IUCN Green List of Protected and Conserved Areas (IUCN 2020).

Between 2005 to 2008, the Nam Theun 2 (NT2) hydroelectric dam, one of the largest dam projects in Southeast Asia, was constructed on the Nam Theun River. To assess the conservation significance of the elephant population on the Nakai plateau and help inform wildlife management strategies to mitigate the impact of the NT2 hydroelectric dam prior to the impoundment of a reservoir on the Nakai plateau, a dung-count based survey and a simultaneous genetic capture-mark-recapture (CMR) study was conducted from February to May 2006 (Ahlering et al. 2011a; Hedges et al. 2013). This study estimated the Nakai population at 141 individuals (95% CI = [95,208]) using the dung-count method and 132 (95% CI = [120,149]) using the genetic CMR method, with 102 unique genotypes identified (Ahlering et al. 2011a; Hedges et al. 2013). The Nakai population's combination of high genetic diversity, largely intact social structure, and

population size identified it as having high conservation value (Ahlering et al. 2011a; Hedges et al. 2013). In subsequent comparisons of the results of the 2006 Nakai study with elephant populations elsewhere in Asia, Ahlering et al. (2020) reiterated the importance of the high levels of genetic diversity found in the Nakai elephant population.

The Nakai plateau underwent major habitat transformation following the closure of the NT2 hydroelectric dam in 2008. Prior to dam closure, most of what became the 450 km² Nam Theun (NT) reservoir was intact forest, and an estimated 40% of suitable elephant habitat on the plateau was lost (McWilliam et al. 2010). Given this major loss of habitat, the objectives of the present study were to determine the impact on the elephants by assessing the genetic diversity and population structure on the Nakai plateau in 2018/19 and comparing them to the 2006 genetic study (Ahlering et al. 2011a; Hedges et al. 2013). To further understand possible patterns of elephant dispersal, we compared our results with those of a 2011 genetic CMR elephant survey (Eggert and Ruiz-Lopez 2012) and a human–elephant conflict (HEC) study (Hedges and Hallam 2011) in the Sepon mine area/Phou Xang He National Protected Area (PXH NPA) approximately 100km south-east of the Nakai plateau in Savannakhet Province. We also reviewed information about the geographic patterns of HEC (and thus elephant occurrence) on the Nakai plateau and in the wider landscape, both before and after the closure of the NT2 dam (McWilliam et al. 2010; Tyson and Rasphone 2013; Tyson and Stremme 2020).

METHODOLOGY

Genetic Analyses – We collected samples on the Nakai plateau from 01 March 2018 – 01 May 2018 (dry season), and from 01 October 2019 – 01 November 2019 (wet

season). Following the recommendations of Hedges and Lawson (2006), we focused our genetic CMR survey efforts near wildlife trails and corridors, mineral licks, locations of recent sighting reports by local fishermen, and other known elephant ‘hotspots’ (Figure 2); the same approach was used for the 2006 genetic CMR survey (Hedges et al. 2013). We also conducted extensive boat-based surveys to search for recent elephant damage to river and stream banks, and thus fresh elephant dung for collection, throughout the network of rivers within the boundaries of NNT NP. An additional 10 samples from 9 adult females and 1 juvenile male were collected, to serve as a known outgroup and as genotype and sexing controls, from a captive population of retired timber elephants in Luang Prabang’s Elephant Village in north-central Laos in April 2018.

From each fresh dung pile sampled, approximately 10 grams of fecal matter was placed in a 40 mL polypropylene tube, and the sample preserved in Queen’s College Buffer (QCB; 20% DMSO, 100 mM Tris pH 7.5, 0.25 M EDTA, saturated with NaCl, Amos et al. 1992). All samples were stored at -20°C prior to export to the United States for genetic analyses. Fresh dung piles were identified using the criteria of Hedges and Lawson (2006). The circumferences of the three largest dung boli in each dung pile were measured to provide an estimation of elephant age (Tyson et al. 2002).

The elephant samples from the 2018/19 survey were compared with those collected during the 2006 genetic CMR survey of the Nakai plateau and a 2011 genetic CMR survey of the Sepon mine area/PXH NPA. Specifically, we used (1) a subset of DNA extractions from the 2006 Nakai plateau survey (19 February 2006 – 01 May 2006), “Nakai 2006”, which had been preserved for long-term storage at -80°C in elution buffer EB (Qiagen) and (2) a subset of the dung samples from the Sepon mine / PXH

NPA survey, “Sepon 2011”, which were collected from 13 January 2011 – 31 March 2011, preserved in QCB, and stored at -80°C.

DNA extraction was completed using a QIAamp Fast DNA Stool mini kit (Qiagen) on the samples from Nakai 2018/19, Elephant Village, and the subset of samples from Sepon 2011 (Archie et al. 2008; Appendix 1). Samples were then amplified at eighteen microsatellite loci (Eggert et al. 2000; Eggert et al. 2008; Kongrit et al. 2008) in 4-5 re-assorted multiplex replicate combinations (Multiplex-A and Multiplex-B) to detect potential pull-up and cross-talk bias (Appendix 2; Appendix 3). Amplification products were verified in a 2.5% agarose gel stained with GelStar (Lonza) and submitted for fragment analysis on an ABI 3730xl DNA analyzer (Thermo Fisher Scientific, Waltham, MA). As an estimate for quality control and null alleles, all samples underwent PCR and fragment analysis at least twice. Genotype scoring was conducted using GeneMarker v.1.9.7 (Holland and Parson 2010).

Nakai 2018/2019 samples were amplified at sex specific fragments: two short Y-specific fragments (SRY1 and AMELY2) and a longer X-specific fragment (PLP1), following Ahlering et al. (2011b; Appendix 2). We also amplified a 593bp mtDNA fragment spanning the C terminal of cytochrome b, the threonine and proline tRNAs, and part of the noncoding control region (Appendix 2). Amplified products were visualized in a 2.5% agarose gel stained with GelStar (Lonza), purified using ExoSap-IT (USB), and sequenced on an ABI 3730xl DNA analyzer (Thermo Fisher Scientific, Waltham, MA). Sequences were trimmed and aligned in Geneious v.8.0.5 (Kearse et al. 2012) and collapsed into haplotypes using FaBox v.1.5 (Villesen 2007).

Genotypes were analyzed for probability of identity (pID) and probability of identity for siblings (pSibs) in GenAlEx v.6.41 (Peakall and Smouse 2012) using the thresholds set by Waits et al. (2001; pID < 0.001; pSibs < 0.01) to identify the necessary minimum number of matching loci to identify individuals. We conducted identity analysis to determine unique individuals and recaptures in Cervus v.3.0.7 (Kalinowski et al. 2007) allowing up to 3 fuzzy matching alleles.

We tested for Hardy-Weinberg equilibrium and linkage disequilibrium in Genepop (Raymond and Rousset 1995; Rousset 2008) for both seasons independently and together. We tested for genetic differentiation by comparing the F_{ST} between seasons using 9 999 permutations in GenAlEx v.6.41 (Peakall and Smouse 2012). Genetic population structure was inferred using the program Structure v.2.3.4 (Pritchard et al. 2000) for up to 10 clusters (K) with 10 replicates for each K. We ran 50 000 burn-in steps and 250 000 MCMC replicates using an admixture model with no inputted priors. We determined the optimal number of genetic clusters using the Evanno method (ΔK ; Evanno et al. 2005) in Structure Harvester v.0.6.94 (Earl and VonHoldt 2012). We tested for population closure for the population both overall and by sampling season in Capture (Otis et al. 1978; White et al. 1982).

We examined social structure by identifying individuals present within a 1 km² sampling area at the same time (association groups) in ArcGIS Pro v.2.7.0 (Environmental Systems Research Institute Inc.) following the methods of Ahlering et al. (2011a). We calculated the average pairwise relatedness within association groups using the Queller and Goodnight (1989) index (r) and assessed whether within-group

relatedness was significantly different than expected using 9 999 permutations in GenAlEx v.6.41 (Peakall and Smouse 2012).

We assessed demographic differences between Nakai 2018 (dry season), Nakai 2019 (wet season), and Nakai 2006 (dry season) by using fecal boli circumferences (as a proxy of elephant age; Tyson 2002) classified by sex and compared between years. We tested for normality of distributions using a Shapiro-Wilks' test for normality and conducted a Mann-Whitney test in Stats v.4.0.3 (R Core Team 2018) to assess differences between 2006 and 2018/19 surveys.

Preserved DNA samples from the Nakai 2006 study were concentrated to 20 μ l using an ethanol precipitation and quantified using a NanoDrop spectrophotometer (Thermo-Fisher Scientific). We selected the 10 best samples for amplification based on a 260/280 ratio closest to 1.80 with a DNA concentration greater than 15 ng/ μ l. Selected samples were amplified and re-genotyped at nine Asian elephant loci used in the original surveys (Ahlering et al. 2011a). The remaining genotypes produced during the Nakai 2006 and Sepon 2011 studies were then calibrated at each locus using the shifts detected from the re-genotyped individuals.

Using the nine nuclear loci shared with the Nakai 2006 survey, and the surveys in Sepon 2011, and Nakai 2018/2019, we calculated expected heterozygosity (H_E), observed heterozygosity (H_O), Shannon's information index (I), and mean fixation index / inbreeding coefficient (F_{IS}) in GenAlex v.6.41 (Peakall and Smouse 2012). To correct for unequal sample sizes among populations, allelic richness (A_R) and private allelic richness (A_P) were calculated using rarefaction in HP-Rare v.1.1 (Kalinowski 2005). We tested for normality of each per locus metric using a Shapiro-Wilks' test for normality and arcsine

square root transformed the per locus H_E and H_O in RStudio v.3.5.2 (R Core Team 2018). Significant differences in diversity between populations were determined using general linear models with locus as a fixed effect using the Lme4 package v.1.1-23 (Bates et al. 2015) with A_R and A_P transformed to gamma distributions. These tests were followed by an ANOVA in the package car v.3.0-9 (Fox and Weisberg 2019) and post-hoc testing using a Tukey test in multcomp v.1.4-13 (Hothorn et al. 2008).

A comparison of the proportion of the total genetic variance contained within each population relative to the total genetic variance (F_{ST}) was completed in GenAlEx v.6.41 (Peakall and Smouse 2012) using 9 999 permutations with a standard Bonferroni correction to determine statistical significance (Neyman and Pearson 1928). Genetic population structure was determined in Structure v.2.3.4 (Pritchard et al. 2000) using the same methodology described previously.

For mitochondrial DNA, we calculated haplotypic diversity (h), nucleotide diversity (π), and determined significant differences using *genetic_diversity_diffs.R* v.1.0.6 (Alexander et al. 2016) with 10 000 permutations. We tested for genetic differentiation (ϕ_{ST}) in Arlequin v.3.5.1.2 (Excoffier et al. 2005), assessing the significance of differentiation using 1 000 permutations and applying a standard Bonferroni correction (Neyman and Pearson 1928).

To complement the genetic studies, we collated and reviewed reports on elephant presence, movements, and dispersal, mainly but not solely relating to HEC, on the Nakai plateau and in the wider landscape encompassing the NNT NPA, the Nakai and Gnommalath Districts of Khammouan Province, and the Sepon mine / PXH NPA areas of

Savannakhet Province (Figure 1; McWilliam et al. 2010; Hedges and Hallam 2011; Tyson and Stremme 2020).

RESULTS

Genetic study of 2018 and 2019 – We collected 125 samples from the Nakai plateau: 84 samples during the dry season in 2018 and 41 during the wet season in 2019 (Figure 2). Analysis of a preliminary subset of samples determined that a minimum of 8 loci was necessary to reach the necessary threshold of distinguishing individuals ($p_{ID} = 0.000$ and $p_{Sibs} = 0.005$; Waits et al. 2001). Two samples failed to amplify at the required minimum number of 8 loci and were removed from further analyses. From the 123 remaining samples, we determined 91 unique individuals; 53 from 2018, 33 from 2019, and 5 captured during both seasons. We recaptured 15 individuals, and within those we had a mean recapture rate of 3.133 ± 0.957 . Capture (Otis et al. 1978; White et al. 1982) determined a lack of population closure overall and for each season. Therefore, we were unable to conduct further analysis of population size, for example through use of CMR methods.

Despite all loci being polymorphic with no evidence of linkage disequilibrium, when we combined the elephants from wet and dry seasons, we found numerous loci did not conform to expectations under Hardy-Weinberg equilibrium (Appendix 3). Further analysis in GenAlEx (Peakall and Smouse 2012) also determined significant genetic differentiation ($p = 0.007$; $F_{ST} = 0.012$) between seasons. We identified three genetic clusters ($K = 3$, $\Delta K = 43.285$) between the wet and dry season, but neither season correlated to a specific cluster.

In Nakai 2018 (dry season), we found 15 adults, 27 subadults, 11 juveniles, corresponding to 34 females and 19 males. In Nakai 2019 (wet season), we found 9 adults, 12 subadults, 7 juveniles comprised of 19 females and 9 males. We also found 4 females and 1 male during the wet season whose age could not be determined due to a lack of intact dung boli. The five individuals found in both seasons consisted of an adult female, two subadult females, and two subadult males.

We found 14 association events in which elephants were found within the same 1 km² block as each other within a 24 hr period, with 9 of these groups having shared individuals. We found an average r of 0.012 (0.018 SE; 95% CI = [-0.169, 0.147]). A decrease in relatedness was found among females in groups to $r = -0.002$ (0.022 SE; 95% CI = [-0.161, 0.152]), and among adult and subadult males, average relatedness also decreased to $r = -0.037$ (0.093 SE, 95% CI = [-0.348, 0.299]).

Comparisons between the genetic studies of 2006 and 2018/19 - We successfully re-genotyped 10 individuals from Nakai 2006 and 9 individuals from Sepon 2011 allowing for a per-locus calibration with the 2018/2019 dataset. Our final calibrated datasets for population comparisons included 58 individuals from the Nakai 2018 dry season, 38 from the Nakai 2019 wet season, 10 from the Elephant Village, 32 from the 2011 Sepon survey, and 102 from the Nakai 2006 dry season survey at the shared 9 microsatellite loci, a mitochondrial fragment, and demographic information.

We found the Nakai 2018 and 2019 populations, combined and separately, to be normally distributed for fecal bolus circumferences (a proxy for age), while the Nakai 2006 dry season population had a bimodal distribution ($W = 0.968$, $p = 0.025$; Figure 3). We found that this was driven by the non-normality of females in Nakai 2006 ($W =$

0.932, $p = 0.046$). Despite the difference in normality, Mann-Whitney tests between populations were non-significant. There was a shift in the sex structure of the population, with the ratio of males to females changing from 1:3 in Nakai 2006 to 1:2 ratio in Nakai 2018 and Nakai 2019.

We found significant declines in nuclear diversity from Nakai 2006 to Nakai 2018/2019 in expected heterozygosity and Shannon's information index (Table 1; Appendix 4; Appendix 5). We also found significantly lower allelic richness in the Nakai 2019 population (ANOVA $p = 0.007$; Tukey 2006 $p = 0.022$; 2018 $p = 0.010$).

For mtDNA, we found 5 of the 6 previously discovered haplotypes from the Nakai plateau. Haplotypes LaoPDR-A, LaoPDR-B, LaoPDR-C, LaoPDR-D are from the Asian elephant α -clade, whereas LaoPDR-E and LaoPDR-F are from the β -clade (Fernando et al. 2000). LaoPDR-A also corresponds to AH of Vidya et al. (2009), LaoPDR-D to AC (Vidya et al. 2009) and LaoPDR-E to NewB haplotype in Thongchai et al. (2011). We found significantly higher nucleotide diversity in Nakai 2018 than Nakai 2006 ($p = 0.001$). This was largely driven by an increase of LaoPDR-E and corresponding proportion of β clade haplotypes from 17% in 2006, to 47% in 2018 (Table 1).

We identified three genetic populations overall ($K = 3$; $\Delta K = 98.657$) broadly identified as Cluster1- Nakai 2006, Cluster2 - Nakai 2018 and Nakai 2019, and Cluster3 - Sepon 2011 with the Elephant village split primarily between Cluster 2 and Cluster 3 (Figure 4). The majority of elephants in Nakai 2018/2019 assigned ($Q \geq 0.50$) to a genetic cluster originally detected at low frequency in Nakai 2006. We found fewer individuals assigning to the Cluster3 in Nakai 2018 than Nakai 2006, and none in Nakai

2019. We found almost all populations to be significantly differentiated based on 9 nuclear microsatellite loci (F_{ST} , Table 2). Based on mtDNA ϕ_{ST} , Nakai 2006 significantly differed from Nakai 2018 ($p = 0.000$), but not Nakai 2019, and Sepon differed from all other populations.

Review of non-genetic studies 2004 – 2019 – HEC on the Nakai plateau and in the surrounding areas were recorded by Wildlife Conservation Society (WCS), District Agriculture and Forestry Office (DAFO), and NTPC staff over the October 2004 to December 2009 period. Prior to the creation of the NT reservoir in April 2008, the majority of the HEC occurred on the plateau, although there were some incidents in one village in Thongkhong area of the Gnommalath plain some 5–10 km south/south-east of the plateau. Since the formation of the NT reservoir, there was a large increase in the number of incidents off the plateau: both (1) in the Thongkhong area, where the number of villages affected increased from one to nine and (2) in the Tha Thod area (Figure 5; Table 3). Prior to the creation of the reservoir, no HEC incidents had been recorded in the Tha Thod area; indeed, HEC only began in Tha Thod area during September 2009, but by December 2009 the area experienced the second highest rate of HEC incidents since monitoring began in 2004. Although there is no direct evidence to show that the elephants involved in HEC in the Tha Thod and Thongkhong areas were from the Nakai plateau, anecdotal evidence does suggest this was the case as there were no other known elephant populations in the surrounding districts. Moreover, HEC incidents had occurred in one Thongkhong village since monitoring began in 2004 and many farmers commented that the elephants involved in these incidents come from the Nakai plateau

using several different tracks to descend off the plateau including the old logging road that connects Khonkhen to Thongkhong (McWilliam et al. 2010).

HEC caused by elephants dispersing from the Nakai plateau also occurred considerably farther away. During fieldwork by WCS staff in the Sepon mine area, some 100km from the Nakai plateau, in January 2011, conversations with farmers (primarily, but not solely in Ban Namkheun and Ban Namalou; Figure 1) indicated that HEC had increased over the previous 2–5 years. Several reasons were given for this reported increase: an increase in disturbance in the PXH NPA including logging and illegal killing of elephants, conversion of saltlicks to rice fields, an increase in the human population in the Sepon area, disturbance caused by the Sepon mine, and the inundation of the Nakai plateau as a result of the closure of the NT2 dam; which villagers said had caused elephants to move off the plateau and into the Sepon area. The reported timing of this last phenomenon coincided with the start of the inundation of the Nakai plateau in 2008 following closure of the NT2 dam (Hedges and Hallam 2011).

In addition, in 2009 a group of three elephants moved away from the Nakai plateau into the Gnommalath plain area and south-west of the plateau and caused serious problems in villages which had no previous history of elephant conflict. The destructive behavior of this “group of three” (G3) elephants became a well-publicized, seemingly intractable problem not least because the elephants were routinely aggressive towards villagers, even during daylight. During 2016, the G3 elephants moved into PXH NPA, and in conjunction with the local and provincial government agencies, NTPC suggested that the elephants be fitted with satellite collars for monitoring purposes, but be left in the NPA since they appeared to have ceased their problematic ranging behavior and HEC

frequency was much lower. By 2019, the elephants which comprise the “G3” expanded to include six individuals (three adult females plus three calves). Based on DAFO and NTPC data, the six elephants were believed to be using an extensive area close to the boundary between Khammouane and Savannakhet Provinces, along the eastern edge of PXH NPA (Figure 1), approximately 100 km southeast of the Nakai plateau (Tyson and Stremme 2020).

DISCUSSION

The building of the NT2 hydroelectric dam and subsequent inundation of the Nakai plateau provided a unique opportunity to study the effects of habitat transformation on an elephant population by comparing genetic samples and data on HEC collected pre- and post-inundation both in the Nakai area and the wider landscape. Along the Mekong River Basin, Laos’ intention to become the “battery of Southeast Asia” has resulted in over 50 hydroelectric dams in 15 years, with a further 101 under construction or planned (Chang 2013; Williams 2019). Numerous hydroelectric dams are also being built elsewhere in the region. Many of these dams are in biodiversity rich areas, and so it is vitally important to understand the impact of such projects on wildlife populations.

In the Nakai plateau population, we found a lack of population closure – necessary for CMR analysis in which neither immigration nor emigration occurs – with significant genetic differentiation between survey seasons in 2018 and 2019, and only five individuals shared between surveys. Ahlering et al. (2011a) speculated that the Nakai Plateau elephant population could be part of a larger metapopulation because of the unusually high levels of genetic diversity found. Specifically, they deliberated whether

rather than being a well-established population long resident in the area, the population was actually comprised of remnants of other populations that had been forced to relocate to the Nakai plateau from other parts of Laos and Vietnam. Our 2018/2019 results could relate to these hypotheses or indicate differences in seasonal usage among individuals in the present elephant population. These seasonally varying individuals are comprised of a relatively large number of subadults representing 53% and 42% of the population in 2018 and 2019, respectively, while subadults accounted for only 31% in the 2006 dry season. There was also a shift in sex ratios as males to females were at a 1:3 ratio in Nakai 2006 and have since increased to a 1:2 ratio in Nakai 2018 and Nakai 2019. In Asian elephants, males are typically the dispersing sex while females exhibit philopatry (Vidya and Sukumar 2005), therefore this shift toward subadult may be indicative of bachelor dispersers investigating the plateau area or loss of female-biased philopatry in the area. NTPC has been replenishing salt lick along the reservoir since 2008, which might also influence the movement of elephant groups on the plateau. Furthermore, the non-genetic data related to the so-called “group of three” elephants (G3) showed female dispersal and thus demonstrated breeding females leaving the Nakai plateau after inundation and establishing new home ranges in the PXH NPA area, some 100km away.

A naive comparison of the 102 individuals known to be in the population in the 2006 dry season to the 58 found in 2018 dry season could suggest a likely significant reduction in population size. While no significant increase in inbreeding was detected, the fewer individuals occupying the area in 2018/2019 could present future problems as generational turnover occurs. The average age of first reproduction in Asian elephants is 13 years (De Silva 2013), yet only 10 years had passed between the completion of the

dam in 2008 and the start of the 2018 survey, therefore the full effects of habitat loss on inbreeding in the current state of the population may not yet be detectable. The significantly lower allelic richness of 2019 from 2006 and 2018 was likely due to the high number of close relatives found in the detected genotypes. We found a single male with 17 first-degree relatives ($r \geq 0.50$); such high reproductive output by a few individuals in a small population can result in a decline in effective population size (Caballero 1994).

The significant loss of nuclear genetic diversity following the construction of the NT2 dam is concerning, as the loss of diversity can affect long-term population viability (Reed and Frankham 2003), particularly for small populations. However, the current level of genetic diversity exhibited by the Nakai plateau elephant population is still comparable to other high diversity populations found in southeast Asia including those in Thailand (Kongrit et al. 2008), Cambodia (Gray et al. 2014), and Myanmar (Kusza et al. 2018). The only diversity metric that increased was nucleotide diversity of mtDNA, however this was a consequence of increasing proportions of β -clade haplotypes into the Nakai plateau, particularly during the 2018 dry season.

To conclude, the completion of the NT2 dam in 2008 and subsequent flooding of much of the Nakai plateau resulted in significant loss of high-quality habitat for the Nakai elephant population, a major increase in HEC locally at least initially (April 2008 through to December 2009), and long-range dispersal by some of the Nakai elephants with the creation of new, serious HEC problems some 100km from the Nakai plateau. While the current size of the Nakai elephant population is not known with certainty, it seems likely that there has been a significant reduction in the size of the population, indeed the

population may now only be about half the size it was in 2006 (102 unique individuals were identified in the 2006 dry season compared with only 58 in the 2018 dry season), which will have significant deleterious effects on the population's viability. Nuclear genetic diversity is already significantly lower than in 2006 and the social- and sex-structure of the population less favorable to long-term viability. Further work on the sizes, connectivity, and metapopulation dynamics of the of the elephant populations throughout Bolikhamxay, Khammouane, and Savannakhet Provinces as a whole should be conducted to better understand their status, conservation significance, and management needs, particularly given the continuing infrastructure development in these areas. Finally, this study provides a greater understanding of not only the direct local impacts of hydropower projects on elephant populations, but also the landscape-level effects of such projects, which to date have been under-appreciated.

ACKNOWLEDGEMENTS

We wish to thank the Wildlife Conservation Society for their work on the Nakai 2006 and Sepon 2011 elephant surveys and HEC work in the Nakai, Sepon, and PXH NPA areas. We also acknowledge the foresight of Drs. Marissa Ahlering and Mari Ruiz-Lopez for preserving samples from the 2006 and 2011 surveys thus allowing for the cross-study calibration of microsatellites. This work would not have been possible without the wildlife survey guides, technicians, and administrative support from the Nam Theun 2 Watershed Management and Protection Authority, Nam Theun 2 Power Company Ltd, Lao Department of Forestry and Agriculture, and Association Anoulak. We thank the managers and field personnel from the District and Provincial Agriculture

and Forestry Offices for HEC data gathering and reporting. We also want to thank the Elephant Village in Luang Prabang for allowing us to sample their captive elephants. This research was funded by the American Philosophical Society Lewis and Clark Field Grant and the TransWorld Airline Scholarship fund. KB was funded by a National Science Foundation Graduate Research Fellowship.

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TABLES

Table 3.1: Mean diversity metrics by population. Nuclear diversity: observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}), Shannon diversity index (I), allelic richness (A_R), and private allelic richness (A_P). MtDNA diversity: individual haplotype distribution (H) with the proportion of α and β evolutionary clade haplotypes ($\alpha : \beta$), haplotypic diversity (h), and nucleotide diversity (π). P-values for significant differences shown and indicated with *

		Nakai 2006 Dry Season	Nakai 2018 Dry Season	Nakai 2019 Wet Season
Nuclear Diversity	H_O	0.667 +/- 0.037	0.597 +/- 0.047	0.587 +/- 0.052
	H_E^* $p = 0.008$	0.745 +/- 0.028* <i>Nakai2018; p = 0.011</i> <i>Nakai2019; p = 0.039</i>	0.663 +/- 0.050	0.677 +/- 0.046
	F_{IS}	0.106 +/- 0.035	0.093 +/- 0.043	0.138 +/- 0.035
	I^* $p = 0.004$	1.633 +/- 0.105* <i>Nakai2018; p = 0.005</i> <i>Nakai2019; p = 0.028</i>	1.389 +/- 0.142	1.432 +/- 0.120
	A_R^* $p = 0.007$	7.646 +/- 0.559	7.756 +/- 0.851	6.639 +/- 0.438* <i>Nakai2006; p = 0.022</i> <i>Nakai2018; p = 0.010</i>
	A_P	0.961 +/- 0.260	0.783 +/- 0.194	0.283 +/- 0.126
MtDNA Diversity	H <div> <div></div> LaoPDR-A <div></div> LaoPDR-B <div></div> LaoPDR-C <div></div> LaoPDR-D <div></div> LaoPDR-E <div></div> LaoPDR-F </div>			
	$\alpha : \beta$	83% : 17%	53% : 47%	71% : 29%
	h	0.740 +/- 0.021	0.699 +/- 0.041	0.745 +/- 0.042
	π^*	0.011 +/- 0.006* <i>Nakai2018; p = 0.001</i>	0.018 +/- 0.009	0.016 +/- 0.008

Table 3.2: Genetic differentiation based on nuclear microsatellite loci (F_{ST}) sub-diagonal, and based on MtDNA (ϕ_{ST}) in the super-diagonal; significance following Bonferroni correction is indicated with *.

	Nakai2018	Nakai2019	Nakai2006	Sepon2011	Elephant Village
Nakai2018	-	0.253	0.072*	0.304*	0.044
Nakai2019	0.011	-	0.024	0.181*	0.156
Nakai2006	0.012*	0.026*	-	0.175*	0.230*
Sepon2011	0.037*	0.043*	0.033*	-	0.541*
Elephant Village	0.059*	0.065*	0.056*	0.024	-

Table 3.3: Number of Human-Elephant Conflict (HEC) incidents and HEC incident rates, by area, before and after creation of the NT2 reservoir (adapted from McWilliam et al. 2010).

Area	<u>Before Reservoir Creation</u> Oct 2004 - May 2006; Jul 2006 - Mar 2008 - 41 Months -			<u>Following Reservoir Creation</u> 1 Apr 2008 - 21 Dec 2009 - 20.68 Months -		
	Number of HEC incidents	Number of villages	HEC incident rate/month	Number of HEC incidents	Number of villages	HEC incident rate/month
Nakai plateau	280	18	6.83	172	8	8.32
Thongkhong area	83	1	2.02	134	9	6.48
Tha Thod area	0	0	0	142	8	6.87
TOTAL	363	19	8.85	448	25	21.66

FIGURES

Figure 3.1: Map of the Central Lao PDR showing the Nakai plateau, the Nakai–Nam Theun National Park, and the Sepon mine / Phou Xang He National Protected Area.

Figure 3.2: Map of the Nakai plateau depicting sampling areas which emphasized saltlicks and known corridor locations. Additional boat surveys were conducted along the rivers within the boundary of the Nakai–Nam Theun National Park.

Figure 3.3: Age distribution density plots for the Nakai 2006, 2018, and 2019 surveys using fecal bolus circumference as a proxy for age. The age class thresholds defined by Tyson et al. (2002) are indicated.

Figure 3.4: Population structure results from Structure 2.3.4 based on 9 nuclear microsatellite loci for $K = 3$ ($\Delta K = 98.657$) with cluster1 in dark grey, cluster2 in medium grey and cluster3 in light grey.

CHAPTER 4

IDENTIFICATION OF CONSERVATION PRIORITY UNITS IN THE ASIAN ELEPHANT, *ELEPHAS MAXIMUS*

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ABSTRACT

The Asian elephant, *Elephas maximus*, is an endangered species with populations spanning south, southeast, and insular Asia. Although 50-60% of Asian elephants are found in India and Sri Lanka, small, fragmented populations are also found throughout southeast and insular Asia. We evaluated the genetic diversity and evolutionary distinctiveness of populations spanning the Asian elephant distribution. We overcame the microsatellite marker selection bias frequently found in comparisons of genetic diversity by using a yardstick calibration population and model and applied predictive modeling to evaluate diversity across the distribution. We found the greatest diversity in the central, and often highly impacted, populations of southeast Asia. We found the greatest distinctiveness among the subspecies designations, particularly Borneo and Sumatra with other notable southeast Asian populations. Overall, we found that the small populations of southeast Asia, albeit fragmented and at high risk of extirpation, contain valuable diversity and distinctiveness and are thus of high value for the preservation of the Asian elephant.

INTRODUCTION

Despite being a symbol of conservation efforts and an icon even among the charismatic megafauna, elephants require active conservation efforts as populations continue to decline across their range. Conservation efforts generally publicize the African savanna elephant (*Loxodonta africana*), although populations of the African forest elephant (*Loxodonta cyclotis*) and the Asian elephant (*Elephas maximus*) each have only 10% of the size of African savanna elephant populations (Gobush et al. 2021; Williams et al. 2020). Populations of Asian elephant in particular are declining at alarming rates across their range, largely due to habitat degradation, urbanization, and fragmentation. Although globally recognized as an endangered species, only 16% of suitable habitat and geographic range is protected (Choudhury et al. 2008; Williams et al. 2020). Today the Asian elephant occupies fragmented portions of its historic range in 13 countries: India, Bangladesh, Bhutan, Cambodia, Lao PDR, Thailand, Vietnam, China, Myanmar, Malaysia, Indonesia, Sri Lanka, and Nepal (Williams et al. 2020). However, 60% of the total population of Asian elephants resides solely in India and the smaller populations throughout southeast Asia continue to decline from habitat loss with some populations already lost (Williams et al. 2020)

Without high genetic diversity, these small isolated populations can quickly become impacted by the negative effects of genetic drift and inbreeding (Frankham 2005). Therefore, actively preserving the genetic diversity of a species can limit inbreeding depression and maintain the species ability to adapt to stressors such as environmental changes (Reed and Frankham 2003).

Genetic studies of Asian elephants, like those of many species of conservation concern, are heavily reliant on the use of nuclear microsatellite loci to evaluate genetic diversity. To date, several studies have investigated genetic diversity in the Asian elephant using microsatellites. However, results of microsatellite studies can be heavily influenced by marker selection bias and varying marker selection, leading to differences in results. The effect of marker selection difference was clearly demonstrated in the isolated populations of Borneo, where Fernando et al. (2003) found extremely low levels of nuclear genetic diversity with an allelic diversity (A) of 1.4 and expected heterozygosity (H_E) of 0.041, primarily using loci from Fernando et al. (2001). In contrast, in their study of the Bornean elephants, Goossens et al. (2016) found a two-fold increase in A to 2.8 and 30% greater H_E (0.34) using a microsatellite set developed by Kongrit et al. (2008). This difference led Goossens et al. (2016) to conclude that “[diversity] values were overstated” in certain populations. Although there have been few such studies that demonstrate differences in measures of genetic diversity within the same population, large differences in genetic diversity between populations are not uncommon, and it is unknown if they stem from actual differences in genetic diversity or from differences in the microsatellite loci used for the study. The most commonly used loci are those characterized by Fernando et al. (2001; the EMX loci) and those characterized by Kongrit et al. (2008; the EMU loci). The underlying discrepancy between diversity estimates may be due to the structure of the microsatellites themselves. While the EMX loci contain complex motifs forming tri- and tetra- nucleotide repeats, the EMU loci consist of di-nucleotide repeat regions. Di-nucleotide repeats have been shown to accumulate mutations at a rate 1.5 – 2.0 times greater than tetra-nucleotides (Chakraborty

et al. 1997); thus, the choice of loci could substantially affect the number of alleles detected per locus and result in drastically different estimates of diversity.

Another key component in identifying populations valuable for conservation is the preservation of unique lineages. As species are constantly in flux and locally adapted populations can represent distinct lineages and subspecies, Moritz (1994) introduced the concept of management units, proposing that genetically differentiated populations should also have a high priority for conservation efforts. In Asian elephants, three subspecies are currently recognized: the Asian mainland elephant or Indian elephant, *E. m. indicus*, the Sri Lankan elephant, *E. m. maximus*, and the Sumatran elephant *E. m. sumatranus*. Additionally, the Bornean elephant, *E. m. borneensis* has been supported from genetic studies (Fernando et al. 2003; Sharma et al. 2018), but awaits further range wide studies prior to designation (Williams et al. 2020). While nuclear microsatellites results cannot be compared between studies without calibration, mitochondrial DNA (mtDNA) sequences are directly comparable and can reflect ancestral maternal lineages and evolutionary patterns. Nearly every population genetic study on the mtDNA of Asian elephants uses the fragment described in Fernando et al. (2000). This 630bp fragment includes a portion of the C terminal of cytochrome b, the threonine and proline tRNAs, and the 5' end of the noncoding control region (d-loop). This mtDNA fragment was used previously to determine the deep-rooted mitochondrial clades in Asian elephants, which were first suggested by Fernando et al. (2000) and Fleischer et al. (2001) and further validated range-wide by Vidya et al. (2009). These mtDNA clades are described as the α -clade persisting primarily in the north-east areas of the range and the β -clade, primarily

found in the south and south-west (including the Indonesian and Malaysian islands) with the two clades overlapping in Myanmar and Thailand.

The primary objective of this research was to ascertain populations for conservation priority in the Asian elephant by identifying distinct lineages and those with high genetic diversity. We accomplish this by first calibrating the previous population genetic studies of the Asian elephant under a population yardstick model to allow for the direct comparison despite marker selection bias and then applying predictive modeling to unstudied regions to gain an understanding of the patterns of diversity and distinctiveness across the species distribution. We further evaluate populations using evolutionary distinctiveness scores of compiled mtDNA sequences spanning their distribution to assess subspecies designations utilizing evolutionary significant unit definitions (Moritz 1994).

METHODOLOGY

Comparison of Genetic Diversity – To create a yardstick population suitable for a yardstick model calibration, we amplified 91 samples from known individuals at 25 microsatellite loci frequently used in previous studies in multiplexed reactions: EMU01, EMU02, EMU03, EMU04, EMU06, EMU07, EMU08, EMU09, EMU10, EMU11, EMU12, EMU13, EMU14, EMU15, EMU17, EMU18 (Kongrit et al. 2008), EMX1, EMX2, EMX3, EMX4, EMX5 (Fernando et al. 2001), LafMS02, LafMS03, (Nyakaana and Arctander 1998), LA4 (Eggert et al. 2000), and FH94 (Eggert et al. 2008). These samples were obtained in a previous population study of the Nakai plateau in Lao PDR, and were confirmed to represent unique individuals with high levels of polymorphisms making them suitable to serve as a yardstick population (Budd et al. *unpublished*).

Polymerase chain reactions (PCRs) were performed in 8 μ l volumes comprised of 2 μ M primer mix, 0.8 mM BSA, and Platinum® Multiplex PCR Master Mix (2X Master Mix, GC Enhancer; Applied Biosystems, Foster City, CA). Thermocycler conditions were as follows: 95°C for 2 min; 40 cycles of 94° C for 30 sec, multiplex specific annealing temperature for 90 sec (Appendix 4.1), 72° C for 1 min; followed by a final extension at 60° C for 30 min. Preparations for all PCRs were conducted in a UV-sterilized hood to minimize the potential for contamination. Negative (no DNA) and positive controls were included with every sample set to standardize scoring and detect contamination of the PCR reagents. Amplification products were verified in a 2.5% agarose gel stained with GelStar (Lonza) and submitted for fragment analysis in an ABI 3730xl DNA analyzer (Thermo Fisher Scientific, Waltham, MA) at the University of Missouri DNA Core facility and with added 500LIZ size standard. The resulting chromatograms were visualized and scored using GeneMarker v.1.97 (SoftGenetics, State College, PA; Holland and Parson 2010).

To allow for a comparison of genetic diversity across the Asian elephant distribution, we compiled data from population studies that shared more than one microsatellite locus with my developed yardstick population (Table 4.1; Appendix 4.2). From these reference populations studies, we collected the population size (N), expected heterozygosity (HX), allelic diversity (AX) and corresponding standard errors (HX_{SE}; AX_{SE}) for loci shared with our dataset.

The yardstick calibration model followed the techniques described by Skrbinsek et al. (2012) using R statistical software v.4.0.3 (R Core Team 2018). For each reference population, we first subset the yardstick population to shared loci using Genepopedit

(Stanley et al. 2017). We then randomly sampled without replacement from the yardstick population for the N of the reference population and calculated the expected heterozygosity and allelic diversity using Adegnet (Jombart 2008; Jombart and Ahmend 2011). After 1000 iterations of the model, we then calculated the mean and standard error for the iterated subset-resampled allelic diversity (ASR; ASR_{SE}) and expected heterozygosity (HSR; HSR_{SE}). We calculated the expected heterozygosity ratio (HR) and allelic diversity ratio (AR) and corresponding standard errors (HR_{SE}; AR_{SE}) following Skrbinek et al. (2012) as:

$$HR = \frac{HX}{HSR} \quad HR_{SE} = \sqrt{HR^2 \cdot \left(\left(\frac{HX_{SE}}{HX} \right)^2 + \left(\frac{HSR_{SE}}{HSR} \right)^2 \right)}$$

$$AR = \frac{AX}{ASR} \quad AR_{SE} = \sqrt{AR^2 \cdot \left(\left(\frac{AX_{SE}}{AX} \right)^2 + \left(\frac{ASR_{SE}}{ASR} \right)^2 \right)}$$

To determine overall spatial patterns of diversity, we applied empirical Bayesian kriging prediction in ArcGIS Pro using empirical transformation and standard circular neighborhood parameters. For the geographic input, we generated the centroid of each population based on the source literature indications. The predictive raster was then masked using the IUCN Asian elephant shapefile (Williams et al. 2020) to the current extent of the Asian elephant distribution.

Evaluation of Distinct Lineages – We compiled mtDNA sequences from published articles, reports, and GENBANK accessions. We continued the naming convention set forth by Vidya et al. (2005a) to the haplotypes described in these additional studies for clarity and to avoid name duplicates such as “New Haplotype” that

were frequently encountered in the literature (original names and corresponding studies are provided in Appendix 4.3) We manually trimmed and aligned sequences using ClustalW (Thompson et al. 1994) within Geneious v.8.0.5 (Kearse et al. 2012). We then collapsed sequences into haplotypes using FaBox v.1.5 (Villesen 2007) and removed duplicate haplotypes. We included a sequence from the Asian elephant's closest known sister taxon, the woolly mammoth *Mammuthus primigenius* (GenBank: KC427957) to serve as an outgroup for analyses. We conducted phylogenetic analyses using maximum likelihood in PAUP* v.4.0a169 (Swofford 2002) using UPGMA with a heuristic search and 1 000 bootstrap replicates to assess support. The DNA substitution model was selected using the automated model selection tool and determined using the optimal AICc. For each haplotype, we calculated evolutionary distinctiveness scores using fair proportions (Isaac et al. 2007) using Picante (Kembel et al. 2010) in R Studio (R Core Team 2018). The visualization into a median joining network (Bandelt et al. 1999) was generated in POPART (Leigh and Bryant 2015) and individual haplotypes were colored to signify source country.

RESULTS

Population Genetic Diversity – We compiled data from 33 populations from India, Laos, Vietnam, Thailand, China, Myanmar, Cambodia, Sumatra, Borneo and peninsular Malaysia (Appendix 4.2). Diversity ratios (HR, AR) are in direct comparison to the yardstick population, for which AR and HR are 1.000. The closer the reference population was to 1.000, the more similar its diversity was to that of the yardstick population, while deviations were interpreted as higher or lower diversity. Among the

reference populations, AR ranged from 0.241 to 1.715 and HR ranged from 0.325 to 1.178. I found the highest ratios of diversity in populations found in Thailand, Myanmar and Laos while populations from China and Borneo contained the lowest diversity (Table 4.1). Analysis of overall genetic diversity based on geographic patterns shows hotspots for expected heterozygosity in southeast Asia and southern India (Figure 4.1). Based on allelic diversity, mainland southeast Asia was the primary diversity hotspot. The lowest relative diversity using each metric were primarily seen in the small populations of Borneo and China (Figures 4.1, 4.2).

Evolutionary Distinctiveness – I compiled mtDNA sequences from populations in 11 home range countries (Fernando and Lande 2000; Fernando et al. 2000; Fernando et al. 2003; Vidya et al. 2005a; Vidya et al. 2005b; Vidya et al. 2007; Vidya et al. 2009; Lei et al. 2011; Ahlering et al. 2011a; Eggert and Ruiz-Lopez 2011; Thongchai et al. 2011; Eggert and Ruiz-Lopez 2012a; Eggert and Ruiz-Lopez 2012b; Sulandari et al. 2012; Elliza et al. 2015; Zhang et al. 2015; Goossens et al. 2016; Eggert and Budd 2017). I found 69 unique Asian elephant haplotypes; 31 from the α -clade, and 38 from the β -clade. Of these 69 haplotypes, only 16 were shared between two or more countries, allowing for 53 private haplotypes. Additionally, two haplotypes were found solely in the zoo population.

The optimal substitution model for building the phylogenetic tree for evolutionary distinctiveness was determined to be HKY+I+G. MtDNA haplotypes with the highest evolutionary distinctiveness scores were found to be AI (0.054; Myanmar), BBG (0.046; peninsular Malaysia), BD (0.036; Borneo), BY (0.031; Laos). BR (0.027; Sumatra), and AP (0.027; zoo only), while the lowest scores were AH (0.000; multiple countries), AB

(0.001; multiple countries), AD (0.001; multiple countries), AR (0.002; Laos), and BF (0.002; India; Figure 4.3). The most widely distributed haplotype was BN, found across 5 countries.

DISCUSSION

The Asian elephant is experiencing alarming decreases in populations across its range. Due to their densely vegetated their habitat, population estimates for Asian elephants can be extremely inaccurate (Blake and Hedges 2004; Hedges 2006). Evaluating the levels and distribution of genetic diversity is vital to elephant conservation, as it allows managers to detect migratory pathways across fragmented landscapes, estimate population sizes and demographic parameters, and infer the proximate and ultimate causes of human-elephant conflict. As has been found previously, the Chinese and Bornean populations had very low diversity, which was especially notable after the data were corrected for marker bias. He et al. (2020) suggested that inbreeding in Chinese populations might be alleviated by establishing connectivity among remnant Chinese populations and with regions such as Laos or by initiating further actions such as translocations to initiate gene flow. On the contrary, Goossens et al. (2016) despite their findings of low diversity in Bornean elephants, expressed concerns with the potential for outbreeding depression if gene flow was established between Bornean and mainland elephants.

Our results support the evolutionary distinctiveness of subspecies such as the Sumatran elephants (*E. m. sumatranus*) and the proposed Bornean elephants (*E. m. borneensis*) using evolutionary significant units and geographic species concepts.

Therefore, the present study underscores Goossens et al. (2016) concerns for the potential of outbreeding depression if translocations are used to enhance genetic variability. However, we also found evolutionary distinctiveness among Indian elephant (aka mainland elephant, *E. m. indicus*) populations, particularly among peninsular Malaysia and Myanmar populations. Although the largest populations of Asian elephant are found in India, we found lower regions of diversity and distinction in these populations than in the small fragmented populations of southeast and insular Asia. The largest Asian elephant population in the world, Nilgiris, is characterized by a single mtDNA haplotype (BN) that is of low evolutionary distinctiveness. In nuclear diversity Nilgiris is also only among the mid-diversity population; lower than those in Thailand, Laos, and Myanmar. The lower diversity found in larger populations in comparison to smaller populations illustrates the necessity of conservation efforts focusing not only on the large populations of India, but also on the smaller populations of southeast and insular Asia for the preservation of diversity and distinction of the species.

Across Southeast Asia, surveys have produced varying estimates of population sizes, especially in Laos (ranging from 500 to 1000; Duckworth and Hedges 1998; Choudhury et al. 2008; Khounboline 2011), Vietnam (100-130 elephants; Williams et al. 2020) and Cambodia (250 to 2000 elephants; Kemf and Jackson 1995; Osborn and Vinton 1999; Murdoch 2008). In Myanmar, where more potential elephant habitat exists than in any other country in the species' range, the total population is estimated at approximately 2,000 individuals, but the country's recent and extreme poaching climate may drastically reduce estimates in coming years (Leimgruber et al. 2011; Sampson et al. 2018). The small populations such as those in southeast Asia that harbor the highest

diversity, are also among those with the highest habitat loss and fragmentation (Williams et al. 2020) and therefore are at high risk for local extirpation. The loss of diversity in southeast Asia would be directly detrimental to the long-term preservation of the species.

The issue of marker selection bias in the comparison of genetic diversity across a species range is not unique to elephants as yardstick calibration models have also been used in studies of brown bears (Skrbinsek et al. 2012) and lions (Miller et al. 2014). However, despite the overwhelming evidence of marker selection bias using microsatellites, few studies address it. Failing to address marker selection bias can lead to problematic results as data from different microsatellite loci may not be directly compatible for comparisons. The downside to yardstick calibration modeling is the need to create a large well studied population that is amplified at all microsatellites in the comparison; which is a costly and time-consuming undertaking. Therefore, I highly suggest that studies, particularly those in conservation, resist the development of new microsatellites as part of their experimental design, but instead continue to use already established and therefore comparable panels. For study species, such as Asian elephants, where separate incomparable panels have dominated the literature, yardstick calibration modeling can identify patterns of diversity.

ACKNOWLEDGMENTS

Thank you to Lauren Sullivan for assistance in developing the yardstick calibration model and Joe C. Gunn for statistical support and manuscript review.

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TABLES

Table 4.1: Comparison of genetic diversity between Asian elephant populations using yardstick calibration allelic diversity ratio (AR) and expected heterozygosity ratio (HR) and the standard error for each (AR_{SE} , HR_{SE}) between the compared population/resampled-correct marker-set specific values for Asian elephants. A diversity value in perfect match with the yardstick population is closer to 1.000, while deviations indicate higher or lower diversity. Additional information on reference populations including sources can be found in Appendix 4.2.

Population		N	AR (AR_{SE})	HR (HR_{SE})
Doi Phamuang	Thailand	64	1.715 (0.183)	1.178 (0.083)
Sublanka	Thailand	34	1.517 (0.158)	1.167 (0.078)
Nakai Plateau	Laos	102	1.013 (0.119)	1.105 (0.075)
Phnom Prich	Cambodia	78	1.206 (0.136)	1.064 (0.095)
Thai Elephant CC	Thailand	83	- (-)	1.063 (-)
Taikkyi	Myanmar	26	0.668 (0.189)	1.031 (0.164)
Bago Yoma	Myanmar	127	0.582 (0.038)	1.002 (0.083)
Myaing Hay Wun	Myanmar	35	0.655 (0.165)	0.988 (0.192)
Maesa	Thailand	53	- (-)	0.965 (-)
Reintro. Found.	Thailand	20	- (-)	0.959 (-)
Eastern Ghats	India	140	0.477 (0.108)	0.953 (0.176)
Nilgiris	India	140	0.477 (0.108)	0.953 (0.176)
Wild-sourced Zoo	USA	164	0.754 (0.134)	0.952 (0.097)
Zoo Elephants	USA	2	0.616 (0.061)	0.945 (0.074)
Seima	Cambodia	55	0.800 (0.104)	0.937 (0.089)
Salak-Phra	Thailand	20	0.591 (0.057)	0.924 (0.079)
Alur Southern	India	29	0.557 (0.066)	0.922 (0.087)
Captive-born Zoo	USA	37	0.641 (0.137)	0.896 (0.110)
Anamalai	India	45	0.559 (0.114)	0.843 (0.174)
Sepon	Laos	31	0.522 (0.051)	0.829 (0.064)
Bukit Tigapuluh	Sumatra	104	0.439 (0.053)	0.826 (0.095)
Periyar	India	27	0.531 (0.122)	0.822 (0.156)
Cat Tien	Vietnam	17	0.339 (0.057)	0.789 (0.144)
Pu'Er-Mengyang	China	49	0.460 (0.063)	0.757 (0.140)
Lower Kinabatangan	Borneo	43	0.506 (0.060)	0.730 (0.072)
Nangunhe	China	24	0.515 (0.129)	0.664 (0.225)
Deramakot	Borneo	33	0.368 (0.040)	0.651 (0.088)
Mengyang	China	55	0.787 (0.208)	0.637 (0.244)
Mengla	China	32	0.578 (0.128)	0.520 (0.136)
Simao	China	8	0.309 (0.047)	0.401 (0.087)

Central Forest	Borneo	127	0.484 (0.055)	0.386 (0.068)
Shangyong	China	59	0.628 (0.263)	0.378 (0.230)
Tabin Reserve	Borneo	21	0.241 (0.032)	0.325 (0.082)
<i>*Yardstick Population</i>		91	1.000(-)	1.000(-)

FIGURES

Figure 4.1: Range wide expected heterozygosity ratio (HR) following Bayesian kriging prediction for the Asian elephant

Figure 4.2: Range wide allelic diversity ratio following Bayesian kriging prediction for the Asian elephant

Figure 4.3: Haplotype network for the Asian elephant 69 mtDNA haplotypes with evolutionary distinctiveness scores shown and colored by source country. Grey circles indicate missing haplotypes that are presumed to exist, but were not sampled.

CHAPTER 5

CONTRIBUTIONS TO THE PRESERVATION OF THE ASIAN ELEPHANT

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CONCLUSIONS AND BROADER IMPLICATIONS

Overall, the purpose of this dissertation is to aid directly in the conservation of the Asian elephant by evaluating direct and indirect human-elephant conflict. The results of these studies can be used to inform decisions that ensure the future of this iconic endangered species and others impacted by human-conflict. Despite the different forms of conflict in both the Nakai plateau and Bago Yoma, I found shared characteristics including displaced elephant populations, loss of female philopatry, and overall disrupted demographic structure as both populations shifted toward age structures dominated by subadults. In females, this shift toward a younger population would lead to the lack of experienced matriarchs and the loss of valuable herd dynamics. The decreased sensitivity to threats (McComb et al. 2011), decreased ability to locate seasonal resources (Foley 2002), and overall younger inexperienced mothers (Douglas-Hamilton and Douglas-Hamilton 1975) in female herds could lead to further declines in survivorship, particularly of juveniles. In males, a decrease in age structure can lead to increased overall aggression (Druce et al. 2006a) primarily due to increased musth frequency and

duration (Druce et al. 2006b) when older dominant males are not present. Furthermore, subadult males, being the primary dispersers of the species, are more likely to investigate new areas (Vidya and Sukumar 2005) and are more likely to engage in crop-raiding (Sukumar 1990; William et al. 2001; Ekanayaka et al. 2011), placing them at further risk for direct human-elephant conflict. In all these situations, the resulting demographic shift could directly contradict elephant conservation efforts.

Specifically, within the Bago Yoma region of Myanmar, my results further suggest that demography could be a key contributor to the high levels direct conflict, particularly crop-raiding. While males are typically associated with conflict behaviors, I also found that females and their juveniles are present. Mitigation strategies against males could present extremely dangerous conditions for juvenile survival. In the local communities of Myanmar, human populations suffer from high levels of crop-raiding and threats to personal safety from elephants, leading to animosity towards wild elephant populations. If human-elephant conflict (HEC) mitigation strategies used by wildlife officials are ineffective at reducing or preventing conflict events, these communities may resort to dangerous HEC mitigation strategies that can exacerbate the current conflict situation (i.e., dangerous AC electric fences, the use of fire crackers) and lead to further declines in Myanmar's wild elephant populations.

The elephants of the Nakai plateau, Laos, once the country's largest population, suffer from indirect conflict in the form of habitat loss and fragmentation. Effective management and monitoring of the short- and long-term reactions of the Nakai elephants to the fragmentation of their habitat could determine the success of elephant conservation in Laos. Elephants are unique in that habitat fragmentation is proceeding at a fast rate in

comparison to their long lifespans and overlapping generations. While their ability to physiologically adapt is limited, the intelligence and social structure of these animals may allow them to teach their offspring successful strategies for acclimating to shifting habitats. My research on the elephants in the Nakai plateau could serve as a model for other studies on wide ranging species on the effects of habitat loss and transformation. With large numbers of hydroelectric dams continuing to be built throughout southeast Asia (Chang 2013; Williams 2019), my results could be informative during the development and planning of projects near biodiversity hotspots such as the Nakai plateau. This is important not only for elephants, but also for human populations that may be impacted by displaced elephants. The results of this project can be used by local management agencies and the government with the assistance of the Nam Theun 2 Power Company in planning for protected area management and wildlife corridors in central Laos. The Nakai plateau population should continue to be surveyed to evaluate the effects of habitat transformation over the long term. This project will aid in managing Asian elephants both now and in the future as hydroelectric dams and human disturbance as a whole continues to heavily impact elephant and other large mammal populations throughout their range.

Conflict with humans continues to be the greatest threat to the conservation of the Asian elephant (Williams et al. 2020). As elephants occupy habitat surrounding some of the world's largest human populations, without the development of proper conservation and management strategies, populations will continue to decline. In regions that have implemented strict conservation protocols, such as in Sri Lanka and the western ghats of India, populations have shown steady improvement (Williams et al. 2020). However, in

regions of southeast Asia, elephant conflict is likely to be underreported and these already small populations have declined significantly. Southeast Asian populations are among the smallest and most highly fragmented remnant populations in the Asian elephant distribution. This dissertation furthermore demonstrated that elephants in regions experiencing high levels of human-elephant conflict, such as habitat loss in the Nakai plateau or the poaching and crop-raiding on the Bago Yoma, are also populations housing some of the highest levels of genetic diversity and unique evolutionary distinctiveness valuable for the species conservation.

FUTURE DIRECTIONS

The field of conservation genetics has grown exponentially in recent years as researchers take advantage of advances in laboratory methods and bioinformatics to analyze population structure and size, habitat use, and movement of individuals in species with low population densities or those that are dangerous or elusive. Non-invasive sampling techniques, specifically the collection of feces, have been used in many species of conservation concern including Bengal tigers (Bhagavatula and Singh 2006), giant pandas (Williams et al. 2016), cheetahs (Gottelli et al. 2007), grey wolves (Stenglein et al. 2011), and African elephants (Eggert et al. 2003; Eggert et al. 2014; Schuttler et al. 2015). While DNA is most easily obtained from blood and tissue samples, trapping and handling large or dangerous animals often requires the use of general anesthetics. For large animals, such as elephants, this places obvious physiological stress on organs, joints, and major blood vessels. Often researchers and veterinary personnel must estimate the amount of anesthetic needed to immobilize the animal long enough to collect

adequate samples. Instead, non-invasive techniques allow for benefits such as maximizing sampling efforts, ensuring the safety of the researcher, and limiting the potentially life-threatening physiological stresses on the study species that are associated with blood and tissue sample collection.

Great strides in the direct application of genetics in elephant conservation using these techniques have been demonstrated by the results of Wasser et al. (2004) in African elephants, in which a broad fecal sampling effort resulted in a database for sourcing illegally obtained ivory. This was a monumental step in African elephant conservation, as regions with high levels of poaching could be identified based on genotypes of confiscated ivory, allowing wildlife authorities to more effectively focus their efforts. While many have considered applying similar genetic methods to Asian elephants, it is likely to be less informative due to the Asian elephant's long history of trade between range states which has resulted in significant mixing of lineages. Thus, sourcing ivory, skin, or other Asian elephant products requires a much more fine-scale method for detecting patterns.

One method to provide the fine-scale analyses necessary to tease apart the complicated history of the Asian elephant is to use information spanning the entire genome, known as genomics. Single nucleotide polymorphisms (SNPs) are the most common variations found throughout the genome of organisms and have been used extensively in studies of model organisms. The advancement to genomics, such as whole genome sequencing, genotype by sequencing (GBS; Elshire et al. 2011), or sequencing following restriction enzyme digestion (ddradseq; Peterson et al. 2012) in Asian elephants, at time of writing, has been largely restricted to captive individuals due to the

current reliance on non-invasive sampling, primarily the collection of feces, to obtain DNA from wild individuals. The problems faced in progressing to genomics was illustrated in Goossens et al. (2016) during their evaluation of Bornean elephants using both blood and fecal samples. While all blood samples were successfully genotyped using single nucleotide polymorphism (SNP) markers, fecal samples had a low percentage of positive PCRs, high levels of allelic dropout (>20%), and genotype error rates up to 25%. Goossens et al. (2016) also determined that the few SNP markers that were confidently obtained from feces were not powerful enough for their population structure assessment and instead relied on microsatellite data for such analyses.

There are two prominent hurdles currently preventing the use of genomics with non-invasive samples: poor DNA quality and environmental contamination. Poor quality occurs as the DNA found in fecal samples is largely restricted to the outermost layer of the fecal bolus; within the sloughed intestinal cells obtained as the digested food passed through the intestinal tract. Once feces been excreted by the animal, the DNA is damaged as it is exposed to the elements and UV radiation. This damaged DNA is difficult to amplify and sequence. In addition, the animal's DNA is not the only genetic material present in these samples. Microbial DNA contaminates every sample, as bacteria from the host's digestive tract vastly outnumber the host's cells. Additionally, direct exposure to the environment places it at an additional risk for contamination from any nearby species, including insects. Current genomic techniques require the use of high-quality DNA with little to no contamination, neither of which are characteristics common to fecal samples.

Although several disciplines continually face the challenges of DNA degradation and contamination, few rival the advancements from ancient DNA studies (Burbano et al. 2010; Carpenter et al. 2013; Castellano et al. 2014; Enk et al. 2014; Gasc et al. 2016). Recent research determining genomes using archeological teeth and bones has paved the way for whole genome analysis of highly degraded and contaminated DNA by using “bait and fish” hybridization (Burbano et al. 2010; Carpenter et al. 2013; Castellano et al. 2014). This technique utilizes a modern human RNA strand as a “bait” system, able to adhere to the genetically similar ancient hominin DNA. It then is able to “fish” degraded hominin DNA from the high contamination “pond”. This baiting system allows for the amplification of entire genomes from highly degraded and contaminated samples. In addition, by using the same “bait” researchers are able to replicate results across laboratories.

Ultimately, this would be the necessary next step in Asian elephant conservation management by providing methodology that advances non-invasive sampling into the genomics era. The proof of concept, the quantification of the error rate, and the documentation of the improvement such an approach provides over traditional genetic methods will constitute major advances in conservation genomics, especially for dangerous, elusive species like elephants. With the development of the genomics methodology with non-invasive sampling, this would provide more reliable and repeatable methods for fecal based surveys, constituting significant advances in the conservation of Asian elephants and other iconic endangered species.

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APPENDIX:

Appendix 3.1: Adaptations to DNA Extraction Methodology –

DNA extraction was completed using a QIAamp Fast DNA Stool mini kit (Qiagen) on samples from Nakai 2018/2019, Elephant Village, and the subset of preserved samples from the Sepon 2011 survey. I adapted the recommendations from Archie et al. (2008) for the new kit version as well as implemented additional modifications.

1. For samples from Nakai2018, Nakai2019 and the Elephant Village, I combined 950mg of fecal sample, 1000µl of InhibitEX buffer, and 50µl of proteinase K in a 2mL microcentrifuge tube. For preserved Sepon2011, this was altered for use with the limited 800mg of preserved fecal sample with additional 100µl of proteinase K and 1100µl of InhibitEX buffer to standardize all samples to 2mL.
2. Incubated samples overnight at 50°C in a rotating mixer at 20RPM
3. Centrifuged samples at full speed for 1 min to pellet stool particles
4. Pipetted 600µl of supernatant into a new 2mL microcentrifuge tube. Pipetted the additional remaining amount of supernatant into a second 2mL microcentrifuge tube taking care not to transfer any solid material.
5. Added 1X volume of Buffer AL to the supernatant tubes (600µl to the first set for the sample), vortex for 15s to form homogeneous solution
6. Incubated all tubes at 70°C for 10 min
7. Added 1X volume of 96-100% EtOH to the lysate, vortex for 15s to form homogeneous solution

8. Applied 600µl of the lysate into the QIAamp spin column and centrifuge at full speed for 1 min. Discard flow through.
9. Repeated step 8 until all lysate from aliquots from same sample have been loaded through the column
10. Added 500µl buffer AW1. Centrifuge 1 min at full speed. Discard flow through.
11. Added 500µl buffer AW2. Centrifuge 3 min at full speed. Discard flow through and collection tube.
12. In a new collection tube, centrifuged the spin column 3 min at full speed.
13. Transferred the QIAamp spin column into a new 1.5mL microcentrifuge tube and pipette 100µl buffer ATE directly onto the QIAamp membrane. Incubate for at least 30min at room temperature, then centrifuge at full speed for 1 min.

Appendix 3.2: Polymerase Chain Reaction (PCR) Conditions –

Microsatellite Multiplexes – Multiplex amplifications were conducted using 1X Platinum Master Mix and GC Enhancer (ABI), 2 mM fluorescently tagged primer mix, and 0.8 mM BSA in 8µl volume reactions. Thermocycler conditions were 95°C for 2 min; 40 cycles of 94°C for 30 sec, 58°C for 90 sec, 72°C for 60 sec; followed by a final extension cycle at 60°C for 30 minutes.

MtDNA Fragments – Amplifications were conducted in 25µl volumes containing 0.4µM forward and reverse primers, 1X PCR Gold Buffer (50 mM KCl, 8 mM Tris-HCl), 0.2 mM dNTPs, 2.0 mM MgCl₂, 0.8 mM BSA, and 0.5 U Amplitaq Gold Polymerase. Thermocycler conditions were 95° C for 10min; 45 cycles of 95° C for 1 min, annealing temperature for 1 min, 72° C for 1 min; with a final extension at 72° C for 10 min.

- MDL3/MDL5 – Annealing temp 60°C, 593bp - spanning the C terminal of cytochrome b, threonine, and proline tRNAs, and part of the noncoding control region (Fernando et al. 2000)
 - MDL3: 5'-CCCACATTAATGGGCCCCGGAGCG-3'
 - MDL5: 5'-TTACATGAATTGGCAGCCAACCAG-3'
- ASDL3/ASDL4 – Annealing temp 55°C, 384bp – overlaps the MDL3/MDL5 fragment into the noncoding control region, (Eggert and Budd 2017). 3' of ASDL4 primer and 3' of MDL3 primer overlap by 7 bp (underlined)
 - ASDL3: 5'-CGGATTGATTTACTTGCAGG-3'
 - ASDL4: 5'-TCCAGTATAGAAACCCCCACAA-3'

Sexing Fragments – Amplifications were conducted in 25µl volumes containing 0.4µM for each of the three primer sets, 1X PCR Gold Buffer (50 mM KCl, 8 mM Tris-HCl), 0.2 mM dNTPs, 2.0 mM MgCl₂, 0.8 mM BSA, and 0.5 U Amplitaq Gold Polymerase. PCR profiles began with denaturation at 95 C for 10 min, followed by 40 cycles of 95 C for 30 s, 59 C for 30 s and extension at 72 C for 45 s. The final step was one cycle at 72 C for 10 min (Ahlering et al. 2011a)

- SRY1 Forward: 5'-CCAGTGGAAAATGCTTACGG-3'
- SRY1 Reverse: 5'-GCATTGCCCTTAGTCTCTGC-3'
- AMELY2 Forward: 5'-CAGGGAGGTTTTACGTTAGGG-3'
- AMELY2 Reverse: 5'-GCGATTTGAAGCTGAAAAGG-3'
- PLP1 Forward: 5'-CCCACCTTCTGCCATATCTGC-3'
- PLP2 Reverse: 5'-GCAGAGGCTCCAACCTCAATC-3'

Appendix 3.3: Per microsatellite locus genetic diversity for the dry season (N = 58), wet season (N = 38), and all individuals (N = 91; five individuals shared between seasons) in Nakai 2018/2019. Microsatellite locus (N = 18), reference, florescent tag label, multiplex reaction number within combination A or B, observed heterozygosity (H_O), expected heterozygosity (H_E), significant p-value (p) following Bonferroni are shown with *.

LOCUS	REFERENCE	TAG	MULTIPLEX		NAKAI 2018			NAKAI 2019			NAKAI 2018 / 2019		
			A	B	H_O	H_E	p	H_O	H_E	p	H_O	H_E	p
EMU01	Kongrit et al. 2008	VIC	3	2	0.636	0.662	0.004	0.528	0.651	0.000*	0.570	0.666	0.000*
EMU02	Kongrit et al. 2008	VIC	4	4	0.345	0.390	0.001*	0.553	0.444	0.486	0.420	0.413	0.006
EMU03	Kongrit et al. 2008	6-FAM	1	4	0.472	0.491	0.007	0.371	0.431	0.040	0.434	0.476	0.002*
EMU04	Kongrit et al. 2008	NED	1	1	0.407	0.428	0.006	0.444	0.518	0.010	0.447	0.492	0.000*
EMU06	Kongrit et al. 2008	6-FAM	3	2	0.545	0.671	0.028	0.353	0.420	0.012	0.500	0.625	0.001*
EMU07	Kongrit et al. 2008	VIC	1	2	0.704	0.847	0.000*	0.618	0.791	0.006	0.663	0.835	0.000*
EMU08	Kongrit et al. 2008	VIC	3	1	0.328	0.428	0.015	0.649	0.581	0.013	0.444	0.498	0.011
EMU09	Kongrit et al. 2008	VIC	4	1	0.558	0.688	0.001*	0.556	0.634	0.068	0.554	0.679	0.000*
EMU10	Kongrit et al. 2008	6-FAM	1	2	0.608	0.555	0.361	0.441	0.632	0.001*	0.550	0.602	0.001*
EMU11	Kongrit et al. 2008	NED	4	4	0.789	0.721	0.091	0.553	0.730	0.003	0.700	0.732	0.006
EMU12	Kongrit et al. 2008	VIC	2	3	0.411	0.634	0.000*	0.486	0.610	0.130	0.443	0.636	0.000*
EMU13	Kongrit et al. 2008	NED	2	3	0.733	0.820	0.052	0.667	0.781	0.268	0.686	0.810	0.002*
EMU14	Kongrit et al. 2008	6-FAM	2	3	0.630	0.756	0.016	0.829	0.791	0.782	0.690	0.781	0.111
EMU15	Kongrit et al. 2008	NED	2	4	0.604	0.651	0.672	0.714	0.720	0.445	0.639	0.676	0.891
EMU17	Kongrit et al. 2008	NED	3	3	0.809	0.787	0.286	0.714	0.822	0.005	0.757	0.805	0.023
EMU18	Kongrit et al. 2008	6-FAM	4	1	0.630	0.626	0.108	0.568	0.636	0.020	0.593	0.630	0.002*
LA4	Eggert et al. 2000	NED	5	2	0.417	0.738	0.000*	0.387	0.770	0.000*	0.392	0.760	0.000*
FH94R	Eggert et al. 2008	NED	5	4	0.340	0.576	0.000*	0.441	0.691	0.000*	0.377	0.640	0.000*
MEAN					0.554	0.637		0.548	0.647		0.548	0.653	
SE					0.036	0.032		0.031	0.03		0.028	0.029	

Appendix 3.4: Diversity values for microsatellite loci ($N = 9$) shared between studies in Nakai 2006, Sepon 2011, Nakai 2018 and Nakai 2019 with per locus observed heterozygosity (H_O), expected heterozygosity (H_E), fixation index (F), Shannon's Index (I), Allelic Richness (A_R) and Private Allelic Richness (A_P)

		EMU03	EMU04	EMU07	EMU10	EMU12	EMU13	EMU14	EMU15	EMU17	MEAN	SE
NAKAI 2006												
	H_O	0.638	0.670	0.838	0.451	0.656	0.619	0.794	0.693	0.645	0.667	0.037
	H_E	0.627	0.746	0.884	0.642	0.674	0.773	0.798	0.758	0.805	0.745	0.028
	F	-0.018	0.102	0.051	0.299	0.027	0.200	0.005	0.085	0.198	0.106	0.035
	I	1.213	1.543	2.324	1.449	1.357	1.698	1.726	1.637	1.755	1.633	0.105
	A_R	5.132	4.996	10.296	7.340	7.497	7.236	8.175	7.349	8.615	7.404	0.488
	A_P	0.557	0.013	0.163	0.157	1.154	0.263	1.224	0.633	1.285	0.605	0.150
SEPON 2011												
	H_O	0.429	0.600	0.806	0.677	0.452	0.724	0.469	0.313	0.621	0.566	0.053
	H_E	0.433	0.529	0.764	0.575	0.617	0.685	0.494	0.303	0.592	0.555	0.045
	F	0.010	-0.133	-0.056	-0.177	0.268	-0.057	0.050	-0.031	-0.048	-0.019	0.042
	I	0.682	0.814	1.507	1.146	1.028	1.229	0.857	0.660	1.104	1.003	0.092
	A_R	3.000	4.000	7.967	5.967	3.968	5.938	6.999	6.967	5.000	5.534	0.494
	A_P	0.000	0.000	0.002	0.295	0.062	0.938	0.000	0.181	0.000	0.164	0.092
NAKAI 2018												
	H_O	0.472	0.407	0.704	0.608	0.411	0.733	0.630	0.604	0.809	0.597	0.047
	H_E	0.491	0.428	0.847	0.555	0.634	0.820	0.756	0.651	0.787	0.663	0.050
	F	0.040	0.049	0.169	-0.095	0.352	0.106	0.167	0.072	-0.028	0.093	0.043
	I	0.976	0.791	1.980	0.977	1.245	1.821	1.613	1.326	1.768	1.389	0.142
	A_R	4.517	5.926	13.055	6.534	8.253	7.729	6.730	6.423	8.362	7.503	0.717
	A_P	0.531	0.657	1.286	0.014	1.594	0.799	0.000	0.020	1.160	0.673	0.177
NAKAI 2019												
	H_O	0.371	0.444	0.618	0.441	0.486	0.667	0.829	0.714	0.714	0.587	0.052
	H_E	0.431	0.518	0.791	0.632	0.610	0.781	0.791	0.720	0.822	0.677	0.046
	F	0.137	0.142	0.219	0.302	0.202	0.146	-0.047	0.008	0.131	0.138	0.035
	I	0.809	1.029	1.774	1.327	1.228	1.608	1.761	1.509	1.847	1.432	0.120
	A_R	4.974	5.790	8.833	7.471	4.965	6.987	6.857	5.811	6.956	6.516	0.375
	A_P	0.196	0.795	0.326	0.074	0.224	0.010	0.000	0.811	0.087	0.290	0.091

Appendix 3.5: Complete p-value table for the diversity metric ANOVA and post-hoc Tukey tests

	ANOVA	Post-Hoc: Tukey Test		
		2018-2006	2019-2006	2019-2018
H _O	0.2091	-	-	-
H _E	0.008*	0.011*	0.039*	0.894
F	0.639	-	-	-
I	0.003*	0.005*	0.028*	0.842
A _R	0.007*	0.961	0.022*	0.010*
A _P	0.349	-	-	-

Appendix 4.1: Multiplex reaction microsatellite loci combinations used to create the yardstick population with source of the loci and florescent tag indicated

Multiplex	Annealing Temp	Loci	Citation	Tag
1	58° C	EMU04	Kongrit et al. 2008	NED
		EMU08	Kongrit et al. 2008	VIC
		EMU09	Kongrit et al. 2008	VIC
		EMU18	Kongrit et al. 2008	6-FAM
2	58° C	EMU01	Kongrit et al. 2008	VIC
		EMU06	Kongrit et al. 2008	6-FAM
		EMU07	Kongrit et al. 2008	VIC
		EMU10	Kongrit et al. 2008	6-FAM
		LA4	Eggert et al. 2000	PET
3	58° C	EMU12	Kongrit et al. 2008	VIC
		EMU13	Kongrit et al. 2008	6-FAM
		EMU14	Kongrit et al. 2008	6-FAM
		EMU17	Kongrit et al. 2008	NED
4	58° C	EMU02	Kongrit et al. 2008	VIC
		EMU03	Kongrit et al. 2008	6-FAM
		EMU11	Kongrit et al. 2008	NED
		EMU15	Kongrit et al. 2008	NED
		FH94	Eggert et al. 2008	PET
5	59° C	EMX2	Fernando et al. 2001	VIC
		EMX5	Fernando et al. 2001	NED
		LafMS02	Nyakaana and Arctander 1998	6-FAM
6	59° C	EMX1	Fernando et al. 2001	6-FAM
		EMX3	Fernando et al. 2001	6-FAM
		EMX4	Fernando et al. 2001	6-FAM
		LafMS03	Nyakaana and Arctander 1998	NED

Appendix 4.2: Comparison of genetic diversity between Asian elephant populations using a yardstick population to correct for different panels of loci and sample sizes. Allelic diversity (AX) and expected heterozygosity (HX) and subsequent standard errors (AX_{SE}, HX_{SE}) for the referenced population were collected, the allelic diversity (ASR) and expected heterozygosity (HSR) from the subset-resampled yardstick population generated with corresponding standard errors (ASR_{SE}, HSR_{SE}) and the comparable corrected diversity ratio generated, allelic diversity ratio (AR) and expected heterozygosity ratio (HR) with standard errors (AR_{SE}, HR_{SE})

Reference Population					Yardstick Population (Subset-Resample)		Diversity Ratio		
Population		Reference	N	AX (AX _{SE})	HX (HX _{SE})	ASR (ASR _{SE})	HSR (HSR _{SE})	AR (AR _{SE})	HR (HR _{SE})
Doi Phamuang	Thailand	Thitaram et al. 2015	64	11.571 (0.779)	0.827 (0.020)	6.748 (0.561)	0.702 (0.046)	1.715 (0.183)	1.178 (0.083)
Sublanka	Thailand	Thitaram et al. 2015	34	11.571 (0.779)	0.831 (0.019)	7.627 (0.605)	0.713 (0.045)	1.517 (0.158)	1.167 (0.078)
Nakai Plateau	Laos	Ahlering et al. 2011b	102	8.100 (0.806)	0.748 (0.024)	8.000 (0.494)	0.677 (0.041)	1.013 (0.119)	1.105 (0.075)
Phnom Prich	Cambodia	Gray et al. 2014	78	6.000 (0.272)	0.668 (0.032)	4.974 (0.512)	0.628 (0.048)	1.206 (0.136)	1.064 (0.095)
Thai Elephant CC	Thailand	Thitaram et al. 2010	83	- (-)	0.597 (-)	3.619 (0.446)	0.562 (0.066)	- (-)	1.063 (-)
Taikkayi	Myanmar	Kusza et al. 2018	26	4.000 (1.020)	0.612 (0.066)	5.985 (0.727)	0.594 (0.069)	0.668 (0.189)	1.031 (0.164)
Bago Yoma	Myanmar	Budd et al. 2021	127	4.725 (0.131)	0.676 (0.031)	8.125 (0.479)	0.675 (0.046)	0.582 (0.038)	1.002 (0.083)
Myaing Hay Wun	Myanmar	Kusza et al. 2018	35	3.800 (0.820)	0.586 (0.091)	5.798 (0.751)	0.593 (0.069)	0.655 (0.165)	0.988 (0.192)
Maesa	Thailand	Thitaram et al. 2010	53	- (-)	0.595 (-)	6.003 (0.656)	0.617 (0.061)	- (-)	0.965 (-)
Reintro. Found.	Thailand	Thitaram et al. 2010	20	- (-)	0.598 (-)	6.953 (0.601)	0.623 (0.061)	- (-)	0.959 (-)
Eastern Ghats	India	Vidya and Sukumar 2005	140	3.500 (0.612)	0.529 (0.073)	7.333 (1.054)	0.555 (0.068)	0.477 (0.108)	0.953 (0.176)
Nilgiris	India	Vidya et al. 2005	140	3.500 (0.612)	0.529 (0.073)	7.333 (1.054)	0.555 (0.068)	0.477 (0.108)	0.953 (0.176)
Wild-sourced Zoo	USA	Lei et al. 2011	164	6.143 (0.891)	0.609 (0.048)	8.143 (0.829)	0.639 (0.041)	0.754 (0.134)	0.952 (0.097)
Zoo	USA	Kongrit et al. 2008	2	4.563 (0.364)	0.612 (0.038)	7.406 (0.429)	0.648 (0.032)	0.616 (0.061)	0.945 (0.074)
Seima	Cambodia	Eggert and Ruiz-Lopez 2012	55	5.250 (0.566)	0.620 (0.048)	6.561 (0.479)	0.661 (0.036)	0.800 (0.104)	0.937 (0.089)
Salak-Phra	Thailand	Kongrit et al. 2008	20	4.188 (0.309)	0.598 (0.042)	7.083 (0.436)	0.647 (0.032)	0.591 (0.057)	0.924 (0.079)
Alur	India	Chakraborty et al. 2014	29	3.800 (0.276)	0.573 (0.033)	6.824 (0.642)	0.621 (0.047)	0.557 (0.066)	0.922 (0.087)
Captive-born Zoo	USA	Lei et al. 2011	37	4.571 (0.854)	0.568 (0.059)	7.134 (0.739)	0.635 (0.042)	0.641 (0.137)	0.896 (0.110)
Anamalai	India	Vidya et al. 2005	45	3.333 (0.451)	0.465 (0.077)	5.965 (0.912)	0.552 (0.068)	0.559 (0.114)	0.843 (0.174)
Sepon	Laos	Eggert and Ruiz-Lopez 2011	31	3.846 (0.296)	0.563 (0.033)	7.370 (0.436)	0.679 (0.035)	0.522 (0.051)	0.829 (0.064)
Bukit Tigapuluh	Sumatra	Moßbrucker et al. 2015	104	3.333 (0.314)	0.551 (0.053)	7.600 (0.562)	0.667 (0.042)	0.439 (0.053)	0.826 (0.095)
Periyar	India	Vidya et al. 2005	27	3.500 (0.612)	0.455 (0.066)	6.592 (0.984)	0.553 (0.068)	0.531 (0.122)	0.822 (0.156)
Cat Tien	Vietnam	Vidya et al. 2007	17	2.333 (0.192)	0.437 (0.059)	6.879 (1.001)	0.554 (0.068)	0.339 (0.057)	0.789 (0.144)
Pu'Er-Mengyang	China	He et al. 2020	49	3.143 (0.315)	0.488 (0.076)	6.834 (0.645)	0.644 (0.064)	0.46 (0.063)	0.757 (0.140)
Lower Kinabatangan	Borneo	Goossens et al. 2016	43	3.300 (0.321)	0.470 (0.040)	6.521 (0.442)	0.644 (0.032)	0.506 (0.06)	0.730 (0.072)
Nangunhe	China	Zhang et al. 2015	24	3.500 (0.750)	0.300 (0.091)	6.797 (0.881)	0.452 (0.069)	0.515 (0.129)	0.664 (0.225)
Deramakot	Borneo	Goossens et al. 2016	33	2.500 (0.218)	0.420 (0.053)	6.790 (0.443)	0.645 (0.032)	0.368 (0.040)	0.651 (0.088)

Mengyang	China	Zhang et al. 2015	55	4.250 (0.893)	0.284 (0.099)	5.402 (0.869)	0.445 (0.071)	0.787 (0.208)	0.637 (0.244)
Mengla	China	Zhang et al. 2015	32	3.750 (0.65)	0.234 (0.050)	6.483 (0.895)	0.450 (0.070)	0.578 (0.128)	0.520 (0.136)
Simao	China	Zhang et al. 2015	8	2.250 (0.217)	0.181 (0.028)	7.286 (0.862)	0.453 (0.068)	0.309 (0.047)	0.401 (0.087)
Central Forest	Borneo	Goossens et al. 2016	127	3.600 (0.354)	0.250 (0.042)	7.438 (0.428)	0.648 (0.032)	0.484 (0.055)	0.386 (0.068)
Shangyong	China	Zhang et al. 2015	59	3.250 (1.244)	0.168 (0.099)	5.174 (0.869)	0.445 (0.073)	0.628 (0.263)	0.378 (0.230)
Tabin Reserve	Borneo	Goossens et al. 2016	21	1.700 (0.202)	0.210 (0.052)	7.060 (0.436)	0.647 (0.032)	0.241 (0.032)	0.325 (0.082)

Appendix 4.3: MtDNA haplotype sequences used to determine evolutionary

distinctiveness scores. The haplotype name used in the present study is shown with the original name and duplicates found in the previous studies of the Asian elephant.

Haplotype	Original Name	Accession	Citation
E _{max} _AA	AA	AY245818.2	Fernando et al. 2003
E _{max} _AAA	05a	KJ187776	Dejchaisri et al. 2009
	MDL15	FJ979512.1	Lei et al. 2011
E _{max} _AAB	07a	KJ187778	Dejchaisri et al. 2009
E _{max} _AAC	11a	KJ187782	Dejchaisri et al. 2009
E _{max} _AAD	15a	KJ187786	Dejchaisri et al. 2009
	15b	KJ187803	Dejchaisri et al. 2009
E _{max} _AAE	17a	KJ187788	Dejchaisri et al. 2009
E _{max} _AB	10a	KJ187781	Dejchaisri et al. 2009
	AB	AY245819.2	Fernando et al. 2003
	MDL12	FJ979441.1	Lei et al. 2011
	Seima-C	-	Eggert and Ruiz-Lopez 2012
E _{max} _AC	03a	KJ187774	Dejchaisri et al. 2009
	AC	AY245815.2	Fernando et al. 2003
	LaoPDR-D	HQ113850	Ahlering et al. 2011b
	MDL13	FJ979611.1	Lei et al. 2011
E _{max} _AD	04a	KJ187775	Dejchaisri et al. 2009
	AD	AY245817.2	Fernando et al. 2003
	LaoPDR-B	HQ113848	Ahlering et al. 2011
	MDL14	FJ979453.1	Lei et al. 2011
E _{max} _AE	14a	KJ187785	Dejchaisri et al. 2009
	AE	AY245822.2	Fernando et al. 2003
E _{max} _AF	AF	AY245827.2	Fernando et al. 2003
	MDL21	AY245827.2	Lei et al. 2011
E _{max} _AG	06a	KJ187777	Dejchaisri et al. 2009
	AG	AY245821.2	Fernando et al. 2003
	MDL20	FJ979569.1	Lei et al. 2011
	Seima-D	-	Eggert and Ruiz-Lopez 2012
E _{max} _AH	13a	KJ187784	Dejchaisri et al. 2009
	AH	AY245820.2	Fernando et al. 2003
	LaoPDR-A	HQ113847	Ahlering et al. 2011b
	MDL17	FJ979457.1	Lei et al. 2011
E _{max} _AI	AI	AY589513.2	Vidya et al. 2009
E _{max} _AJ	AJ	AY589515.2	Vidya et al. 2009

	Seima-B	-	Eggert and Ruiz-Lopez 2012
Emax_AK	AK	AY589516.2	Vidya et al. 2009
	MDL16	FJ979610.1	Lei et al. 2011
Emax_AL	LaoPDR-C	HQ113849	Ahlering et al. 2011b
Emax_AM	12a	KJ187783	Dejchaisri et al. 2009
	NewA1	-	Thongchai et al. 2011
Emax_AN	16a	KJ187787	Dejchaisri et al. 2009
	MDL18	FJ979612.1	Lei et al. 2011
Emax_AO	MDL19	FJ979496.1	Lei et al. 2011
Emax_AP	MDL22	FJ979631.1	Lei et al. 2011
Emax_AQ	08a	KJ187779	Dejchaisri et al. 2009
	MDL23	FJ979634.1	Lei et al. 2011
Emax_AR	NEWH1	-	Eggert and Budd 2017
Emax_AS	NEWH2	-	Eggert and Budd 2017
Emax_AT	NEWH3	-	Eggert and Budd 2017
Emax_AU	NEWH4	-	Eggert and Budd 2017
Emax_AV	NEWH5	-	Eggert and Budd 2017
Emax_AW	NEWH6	-	Eggert and Budd 2017
Emax_AX	NEWH7	-	Eggert and Budd 2017
Emax_AY	01a	KJ187772	Dejchaisri et al. 2009
Emax_AZ	02a	KJ187773	Dejchaisri et al. 2009
	02b	KJ187790	Dejchaisri et al. 2009
Emax_BA	BA	AY365432.2	Vidya et al. 2009
Emax_BB	BB	AY365433.2	Vidya et al. 2009
Emax_BBA	Hap03	-	Elliza et al. 2015
Emax_BBB	Hap05	-	Elliza et al. 2015
Emax_BBC	Hap06	-	Elliza et al. 2015
Emax_BBD	Hap07	-	Elliza et al. 2015
Emax_BBE	Hap08	-	Elliza et al. 2015
Emax_BBF	Hap09	-	Elliza et al. 2015
Emax_BBG	Hap10	-	Elliza et al. 2015
Emax_BBH	02b	KJ187791	Dejchaisri et al. 2009
Emax_BBI	05b	KJ187793	Dejchaisri et al. 2009
Emax_BBJ	07b	KJ187795	Dejchaisri et al. 2009
Emax_BBK	09b	KJ187797	Dejchaisri et al. 2009
Emax_BBL	15b	KJ187803	Dejchaisri et al. 2009
Emax_BC	BC	AY589512.2	Vidya et al. 2009
	MDL8	FJ979531.1	Lei et al. 2011
Emax_BD	BD	AY245538.2	Vidya et al. 2009
Emax_BE	BE	AY245802.2	Vidya et al. 2009
Emax_BF	BF	AY245823.3	Vidya et al. 2009
	MDL1	FJ979503.1	Lei et al. 2011
Emax_BG	14b	KJ187802	Dejchaisri et al. 2009

	BG	AY245824.2	Vidya et al. 2009
	MDL5	FJ979633.1	Lei et al. 2011
Emax_BH	13b	KJ187801	Dejchaisri et al. 2009
	BH	AY245803.2	Vidya et al. 2009
	MDL6	FJ979436.1	Lei et al. 2011
Emax_BI	BI	AY245805.2	Vidya et al. 2009
	MDL4	FJ979517.1	Lei et al. 2011
Emax_BJ	BJ	AY245809.2	Vidya et al. 2009
Emax_BK	BK	AY245810.2	Vidya et al. 2009
Emax_BL	BL	AY245804.2	Vidya et al. 2009
	MDL7	FJ979514.1	Lei et al. 2011
Emax_BM	12b	KJ187800	Dejchaisri et al. 2009
	BM	AY245806.2	Vidya et al. 2009
Emax_BN	06b	KJ187794	Dejchaisri et al. 2009
	BN	AY245826.2	Vidya et al. 2009
	MDL9	FJ979440.1	Lei et al. 2011
	Sepon2	-	Eggert and Ruiz-Lopez 2011
Emax_BO	08b	KJ187796	Dejchaisri et al. 2009
	BO	AY245808.2	Vidya et al. 2009
	MDL11	FJ979439.1	Lei et al. 2011
	Seima-A	-	Eggert and Ruiz-Lopez 2012
Emax_BP	03b	KJ187791	Dejchaisri et al. 2009
	BP	AY245813.2	Vidya et al. 2009
	MDL2	FJ979632.1	Lei et al. 2011
Emax_BQ	01b	KJ187789	Dejchaisri et al. 2009
	BQ	AY245816.2	Vidya et al. 2009
	Hap01	-	Elliza et al. 2015
	MDL3	FJ979606.1	Lei et al. 2011
Emax_BR	BR	AY245811.2	Vidya et al. 2009
Emax_BS	BS	AY245807.2	Vidya et al. 2009
Emax_BT	BT	AY245825.2	Vidya et al. 2009
Emax_BU	BU	AY245812.2	Vidya et al. 2009
	Hap04	-	Elliza et al. 2015
Emax_BV	BV	AY245814.2	Vidya et al. 2009
Emax_BW	BW	AY589514.2	Vidya et al. 2009
Emax_BX	04b	KJ187792	Dejchaisri et al. 2009
	LaoPDR-E	HQ113851	Ahlering et al. 2011b
	MDL10	FJ979616.1	Lei et al. 2011
	NewB	-	Thongchai et al. 2011
Emax_BY	LaoPDR-F	HQ113852	Ahlering et al. 2011b
Emax_BZ	Hap02	-	Elliza et al. 2015

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VITA:

Kiristin (Kris) Ranson was born September 16th 1992 in Fort Hood Texas to Randell and Jodi Ranson. After a childhood spent in a military family, they settled down in the mountains of Heber City, Utah. As part of growing up in the Wasatch back, she explored, hiked, and fished much of the Wasatch back and Uinta mountains, and developed a love for the expansive lands and the wildlife within it. After graduating from Wasatch High School, Kris attended Southern Utah University beginning in 2010. In 2013, she married her college sweetheart, Spencer Budd. At Southern Utah University she specialized in population biology conducting research on the mating systems of the American crocodile, *Crocodylus acutus*, with Dr. Laurie Mauger. Serving as the SUU genetic specialist, Kris also worked projects on a variety of species such as red foxes (*Vulpes vulpes*), grey foxes (*Urocyon cinereoargenteus*), kit foxes (*Vulpes macrotis*), ringtail cats (*Bassariscus astutus*), and Franklin's penstemon (*Penstemon franklinii*). Kris further expanded to local wildlife systems working as a wildlife technician for the intergovernmental internship cooperative and Dixie National Forest. As part of the wildlife team, Kris worked to monitor sensitive and endangered forest species such as the Utah prairie dog (*Cynomys parvidens*), Mexican spotted owl (*Strix occidentalis lucida*), sage grouse (*Centrocercus urophasianus*), and Arizona willow (*Salix arizonica*) as well as forest health indicator species such as the Northern Goshawk (*Accipiter gentilis*) and Northern Flicker (*Colaptes auratus*).

In 2015, Kris graduated with a bachelor's of science in biology with a minor in chemistry from Southern Utah University with honors (*magna cum laude*). She further joined the Eggert lab for conservation and ecological genetics at the University of

Missouri that same year. As an NSF graduate research fellow, Kris conducted her dissertation research on human-elephant conflict with the Asian elephant, *Elephas maximus*, while also collaborating on additional projects such as white-tailed deer (*Odocoileus virginianus*) reintroduction success, elk brain worm infection (*Parelaphostrongylus tenuis*), and African elephant (*Loxodonta africana* and *L. cyclotis*) gut microbiome. Kris also served as a teaching assistant for numerous undergraduate courses including introductory biology, ecology and ornithology. Kris also served as the resident Geographic Information Systems (GIS) collaborator for numerous labs throughout the Division of Biological Sciences. During this time, she also started a 501c3 nonprofit dog rescue, MO Wiggle Butts, and established the last litters program now found in central Missouri.

Following the completion of her dissertation work in 2021, Kris has gone on to pursue a career in wildlife and habitat management. Kris continues to love exploring the outdoors and can frequently be found there with her husband, Spencer, and their three rescue dogs; Mia, Hogan and Nigel.