PRESERVING THE OPPORTUNITY TO EXPAND: CONSERVATION IMPLICATIONS OF ANCIENT DNA

by

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ABSTRACT

The application ancient genetic information to management practices can provide a critical understanding of species of conservation concern. Utilizing the interpretations from two ancient DNA datasets I assess conservation implications for the locally threatened caribou (*Rangifer tarandus*) and the globally endangered saiga antelope (*Saiga tatarica*). Using Bayesian Inference to assess herd affinity of ancient caribou, I identify a dynamic history, including an unexpected lineage replacement event coincident with the deposition of the White River tephra (~1,000yrsBP). I then combine a recently published saiga aDNA dataset identifying a 65-75% population decline likely related to the glacial-interglacial transition at the Pleistocene-Holocene boundary with recent observations of frequent periods of sudden die-off to imply a life history inherently susceptible to dramatic population swings. Accordingly, conservation strategies for these two dynamic northern species must acknowledge both the likelihood of sudden declines, and the necessity for expansion and recovery.

Keywords: ancient DNA; caribou; saiga; conservation genetics; herd fidelity; phylogeography

Subject Terms:
Phylogeography, population genetics, conservation

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CHAPTER 1. INTRODUCTION

Paleontological information has been invaluable to our understanding of the natural world. This information has been applied to a wide variety of applications from exploring the origins of life (e.g., Brocks & Butterfield 2009) to understanding the nature and implications of mass extinction events (e.g., Jablonski 1999), and inferring evolutionary diversification rates (e.g., Quental & Marshall 2010) or reconstructing paleoclimate (e.g., Bunbury & Gajewski 2009). In all, this information has provided a clear insight into the dynamic nature of history (e.g., Drummond et al. 2005; Shapiro et al. 2004), the resilience of the biosphere (e.g., Erwin 1998), and the complex interactions between biotic and abiotic realms (e.g., Campos et al. 2010b). Having a paleontological or long-view perspective on the dynamic nature of nature may be increasingly important in light of the dramatic impacts of anthropogenic activity. As we grapple with the implications of our own actions, a detailed understanding of paleobiological responses to biotic and abiotic events should be important to considerations of the challenges and our responsibilities related to them (Jablonski 2008).

The Pleistocene and Holocene (~2 Ma - to present) provide an excellent source of pertinent information. In part this is because many species present today survived the dramatic glacial-interglacial cycles observed in the Pleistocene (see, e.g., Anderson 1984). As well, due to the relatively short duration of time between Late Pleistocene and the present, we are able to obtain

more detailed information, in particular through the isolation and amplification of ancient genetic material (aDNA; reviewed in Nicholls 2005). This ancient material can then be used to infer major population changes through periods of known climatic upheaval (Shapiro et al. 2004), or to help understand phylogeographic relationships, again with a specific temporal component (Shepherd et al. 2005).

Herein, I discuss the importance of ancient genetic information for two iconic, endangered, and evolutionarily distinctive northern species: caribou (*Rangifer tarandus*) and Saiga antelope (*Saiga tatarica*).

Caribou are a monotypic genus and are classified as of least concern according the IUCN Red List 2010 (Hentonen & Tikhonov 2008). However, this global categorization masks the regional challenges facing many small and ecologically distinctive caribou populations (das Neves et al. 2010). For example, in North America many of the forest-dwelling caribou populations are in decline (Thomas & Gray 2002). According to the Canadian threat classification system, these forest-dwelling caribou are classified as threatened (in large part due to habitat degraedation and climate change; COSEWIC 2002; COSEWIC 2004).

Saiga antelope are considered Critically Endangered under the IUCN Red List 2010 classification (Mallon 2008). In addition, this monotypic genus was ranked 62nd among all mammals using a score of threat and genetic distinctiveness (Isaac et al. 2007).

Here, I consider several bayesian statistical approaches to the analysis of aDNA that can shed light on the modern management and conservation of these two species. I focus on examining the dynamic history of northern mountain caribou herds during the Holocene using Bayesian phylogenetic inference and spatial analysis of molecular variance, combined with detailed analysis of modern herd relationships using Bayesian population clustering. I then re-evaluate the conservation implications for Saiga antelope from a recently published aDNA publication which makes use of Bayesian serial coalescent simulations to identify cryptic population changes (Campos et al. 2010a).

The work presented in the two subsequent chapters have included in two papers in *Molecular Ecology*, volumes ### & **19**, 2010. All work presented in this thesis represents work done by myself, but one should consult the published works for a full account of these collaborative projects. Appendix 1 includes a published book chapter work resulting from collaboration with AØ Mooers, SJ Goring & S Turvey. For this book chapter, I did all the data collection and data analyses, and helped frame and write the manuscript.

CHAPTER 2. CARIBOU OF THE SOUTHERN YUKON

2.1. Introduction

Conservation and management are often focused on species or populations that have small population sizes or have been significantly impacted by recent events, both of which are likely to produce biases in estimates of modern genetic diversity and its inferred ancestry (Leonard 2008). As a result, utilizing the historical perspective afforded by ancient DNA (aDNA) can contribute significantly to the accurate characterization of historic population sizes, levels of gene flow and inter-population relationships (Ramakrishran *et al.* 2005) — contributions which in turn are critical to appropriate conservation and management decisions. Here, we utilize combined modern microsatellite (short tandem repeats, STR) as well as modern mitochondrial DNA (mtDNA) markers and ancient mtDNA recovered from alpine ice patches to investigate historic inter-population relationships and levels of gene flow for caribou (*Rangifer tarandus*) in northern Canada.

Caribou (*Rangifer tarandus*) are an iconic holarctic species, representing one of the last remnants of the Beringian megafauna (Anderson 1984; Kurtén & Anderson 1980), and the last remaining large-scale ungulate migration in the northern hemisphere (Berger 2004). The continued presence of caribou throughout their range is under threat; Vors and Boyce (2009) describe a holarctic synchronous population decline of nearly 60% from recent observed

population maxima in 34 of 43 major caribou populations for which data are available. The authors attribute this unprecedented *synchronous* decline to anthropogenic habitat modification and climate warming, demonstrating the fragility of many of these large caribou populations. In North America, the outlook for caribou is grim, in particular for the forest-dwelling woodland caribou, *Rangifer tarandus caribou* (COSEWIC 2002; Hebblewhite et al. 2007; James et al. 2004; James & Stuart-Smith 2000; McDevitt et al. 2009; McLoughlin et al. 2003; Post & Forchammer 2008; Schaefer 2003; Webber & Flanigan 1997; Wittmer et al. 2005).

Forest-dwelling woodland caribou, hereafter referred to as forest-dwelling caribou, are a paraphyletic subspecies of *Rangifer tarandus*. They are almost exclusively found in Canada, as local extirpations have all but removed them from the southern extreme of their range in the United States. In Canada, forest-dwelling caribou are further classified into one of four federally recognized and at-risk ecotypes: Atlantic, boreal, southern mountain and northern mountain (Thomas & Gray 2002). Northern and southern mountain caribou are distinguished based on their winter diets (terrestrial versus arboreal lichens, respectively; Bergerud 2000; Thomas & Gray 2002). The relict Atlantic caribou populations are listed as endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2002), while both southern mountain and boreal caribou populations are listed as threatened. Northern mountain caribou are classified as being of special concern (COSEWIC 2002), with many populations appearing to be stable or increasing (e.g., COSEWIC 2002; Farnell

et al. 1998; Farnell & Gardner 2003; Farnell et al. 2004). Northern mountain herds exist as small, isolated, sedentary populations. Within our study region in the southern Yukon, northern mountain herds range in size from 235 to 3,750 individuals for the Kluane and Tay River herds, respectively (Yukon Environment Geomatics 2008), with the majority of herds within our study region having populations of less than 1,500 (10 of 16 herds, Yukon Environment Geomatics 2008). This small population size coupled with the remoteness of their habitat, and several decades of active management (Boertje & Gardner 2000; Farnell et al. 1998; Farnell & Gardner 2003; Farnell & MacDonald 1988; Farnell & MacDonald 1989; Farnell & Russell 1984; Southern Lakes caribou recovery program 1996; Thomas & Gray 2002) likely underlie the observed stabilizing trend; many herds were in decline prior to management intervention. Notwithstanding current trends, recent work has suggested that these isolated northern populations may be particularly vulnerable to the rapidly changing arctic climate (Post & Forchammer 2008; Schaefer 2003; Vors & Boyce 2009; Webber & Flanigan 1997; Weladji et al. 2003).

Caribou conservation and management practitioners have called for research on the temporal persistence of caribou herds, which may provide key information to ensure their long-term survival (Farnell *et al.* 2004). A temporal perspective is also crucial for dealing with the large natural fluctuations in population size that characterize many caribou herds (Boertje & Gardner 2000; Kuzyk et al. 1999). Here, we address these questions using mtDNA isolated from caribou that lived in the southwest Yukon over the last 6,000 years in

combination with recent microsatellite (short tandem repeats, STR) work examining modern southern Yukon caribou herds (Kuhn et al. 2010). This combination of genetic markers makes it possible to investigate both short and medium term changes in the genetic structure and diversity of northern mountain caribou in the southern Yukon.

2.2. Materials

2.2.1. Ice patch samples

Ancient caribou remains were first discovered in 1997, when researchers observed extremely high concentrations of caribou fecal material preserved in the alpine Thandlät ice patch, in the southwest Yukon (Figure 1; Kuzyk et al. 1999). Fecal material from the base of a 1.6m ice core was radiometrically dated at 2450±50 yrsBP, opening up the possibility of recovering genetic material from ancient caribou herds (Kuzyk et al. 1999). Since 1997, 72 ice patches in the southwest Yukon have been surveyed by air and ground, with 35 yielding significant biological and archaeological remains (Farnell et al. 2004).

Two subspecies of caribou are known to have recently occupied the region encompassing these ice patches – the migratory Grant's caribou (Fortymile herd), and several sedentary forest-dwelling caribou herds (the Wolf Lake, Ibex, Carcross, and Aishihik herds; Figure 1). The Fortymile herd, which numbered over 500,000 individuals as recently as the late 19th century, ranged between Fairbanks, Alaska and Whitehorse, Yukon (Boertje & Gardner 1996) and would have occupied its southwestern range limit during the winter, when

migratory herds are most dispersed (Farnell et al. 2004; Gunn & Miller 1986; Kuzyk et al. 1999). The sedentary forest-dwelling caribou would have remained present in these regions during the summer months, when woodland caribou undergo modest altitudinal migrations into alpine regions and congregate on alpine ice patches to avoid insect harassment and to aid in thermoregulation (Farnell et al. 2004; Farnell & Russell 1984; Kuzyk et al. 1999).

We sampled forty caribou bone and teeth samples from thirteen ice patches for control region mtDNA (Figure 1; Appendix 1). Twenty-eight of the caribou remains were radiometrically dated as part of an independent study on caribou heavy metal contaminants, with ages for the remaining twelve samples estimated using the distribution of dated caribou samples from the relevant ice patches (G. Hare, personal communications; Appendix 1). All dates, unless explicitly referred to as *calendar* years before 1950 (*cal*-yrsBP) are reported as uncalibrated ^{14}C years before present (yrsBP).

2.2.2. Modern samples

Forty-two samples from the modern Fortymile, Aishihik, Wolf Lake, Carcross and Ibex caribou herds were selected for comparison with ancient mtDNA (Figure 1). These were chosen as the most likely modern relatives under the proposed competing scenarios for southwest Yukon caribou history (see hypotheses below). Modern samples were obtained as extracts, originally isolated from caribou tissue samples, from the Parks Canada DNA Repository (PCDNAR), University of Alberta, Edmonton, Alberta (Appendix 1). To augment

this data set, 7 additional unique control region sequences were obtained from GenBank (Appendix 1).

2.3. DNA extraction and authentication

The ancient caribou remains used in this study have effectively been frozen in the ice patches from the time of deposition to the time of sampling, and as such should be characterized by exceptional DNA preservation. As with all ancient DNA research, however, extreme care is required in order to avoid contamination by modern DNA and/or the potentially complicating effect of DNA damage-induced postmortem mutations. Consequently, protocols designed to minimize such problems were implemented at each step of the extraction and amplification process.

Ancient samples were processed in batches of 6, with appropriate extraction and PCR negative controls, all of which were sequenced. Extraction and amplification of ancient mtDNA was carried out either in the geographically isolated ancient DNA facilities at the Henry Wellcome Ancient Biomolecules Centre (ABC), University of Oxford, or at the University of Alaska Museum of the North (MotN) (Appendix 1). Laboratory work on ancient caribou was completed prior to commencement of modern work. All extractions performed at the ABC used a phenol-chloroform extraction protocol as described in Shapiro et al. (2004). To avoid potential surface contaminants, the surface of each sample was physically abraded using a Dremel cutting tool (Dremel International). New discs were used for each sample. A 0.2 – 1.0g subsample was homogenized using sterilized stainless steel containers and a Braun Mikrodismembrator U (B.

Braun Biotech International, Germany). Following surface decontamination and homogenization, the samples were decalcified overnight in 15ml of 0.5M EDTA (pH 8) solution. The liquid and solid phases were separated by centrifugation, and the liquid phase was discarded. The solid phase was added to 6mL of extraction buffer solution (10mM Tris-HCL, 10mM NaCl, 0.5mg/mL Protenaise-K, 10mg/mL dithiothreitol (DTT), 1% sodium dodecyl sulfate (SDS)) and incubated overnight at 55°C. Final purification of the genetic material involved washing twice with 6mL of Tris-saturated phenol and a final wash with 6mL chloroform. The cleaned extracts where filtered through Centricon centrifugal filters (Millipore Corporation) and the residue was then washed three times with water, and diluted to a final volume of 250µL.

Samples prepared at MotN were collected from each bone by cleaning the surface with a Dremel cutting tool and removing a small piece of bone. New cutting disks were used for each sample. When the bone was a mandible with intact teeth, a tooth was removed and a portion of the tooth cut for sampling. Samples were crushed to a coarse powder with a mortar and pestle. Prior to extraction, 0.5 g of bone powder was decalcified by rotating in 1.0 ml of 0.5M EDTA overnight. After centrifugation, the liquid phase was discarded. 700 µl of buffer solution (10 mM Tris-HCl, 10 mM NaCl, 1% SDS, 0.5 mg/ml Proteinase K and 10 mg/ml DTT) was added to the solid phase and incubated with agitation overnight at 37^{O}C. DNA was recovered using a Qiagen QIAquick Nucleotide Removal Kit ™ with all the extraction buffer being filtered through the spin column in a series of six centrifugations. The collected DNA was eluted from the

filter with 75 μl 10mM Tris and stored at -20^{O}C. During all extractions negative controls and at least one sample of a different species were run to verify the lack of cross contamination of samples.

ABC extracts were prepared for PCR amplification with 4 overlapping primer pairs (Table 1, Gravlund et al. 1998). Thermal cycling and further downstream processing was conducted in a physically isolated laboratory in the Department of Zoology. PCR amplification used a 25μL solution with 2-4μL of extract, 2mg/mL rabbit serum albumin (Sigma), 1.25 U Platinum *Taq* HiFidelity, 250μM each dNTP, 2mM $MgSO_4$, 1x buffer (Invitrogen Ltd., UK), and 2μL each primer. Thermal cycling reactions were 94°C for 90 seconds, followed by 45 cycles of 94°C denaturation for 45 seconds, annealing for 45 seconds and 68°C extension for 120 seconds. Upon completion of 45 cycles a final 68°C extension of 10 minutes was used. Specific annealing temperatures are reported in Table 1.

MotN extracts were amplified in a 2 step procedure using five pairs of overlapping primers designed from modern *Rangifer* sequences (Table 1). After preparation of the first stage PCR, samples were all handled in the West Ridge Research Building at the University of Alaska Fairbanks. The first stage PCR was a 25 μl reaction with 10 μl of 5Prime HotMasterMix™, 0.2 μM of each primer and 2.0 μl of the DNA extract. Thermal cycling conditions were 95^{O}C for 30 seconds followed by 60 cycles of 95^{O}C for 40 seconds, 55^{O}C for 40 seconds and 72^{O}C for 45 seconds. PCR products were visualized in 1.5% agarose gels stained with ethidium bromide. Positive products were cut from the gel and

diluted with 100μl H_2O. These products were melted and 1.5 μl were used in a second 50 μl PCR reaction with 20 μl 5Prime MasterMix™ and 0.2 μM of each primer. Thermal cycling conditions for these reactions were 95^{O}C for 30 seconds followed by 40 cycles of 94^{O}C for 25 seconds, 47^{O}C for 30 seconds and 72^{O}C for 35 seconds. Positive products from these reactions were also cut from 1.5% agarose gels and purified with the MoBio UltraClean™ GelSpin™ DNA Extraction Kit.

Modern DNA extraction and amplification was conducted in the Department of Zoology, University of Oxford. Modern samples received from Parks Canada DNA Repository (PCDNAR) were shipped as extracts, which were used directly in further PCR amplification and sequencing. PCR reactions were run in 25μL solutions with 2μL extract. The solution is as described above (ABC), except RSA was not used, $MgCl_2$ was used in place of $MgSO_4$, and standard *Taq* was substituted for *HiFi Taq*. Thermal cycling was performed for 35 cycles, with the annealing temperature at 57°C, and the extension temperature at 72°C and cycle extension time shortened to 45 seconds. All other parameters were similar to those mentioned for ancient samples from ABC.

Sequencing was performed on both the forward and reverse strand using PRISM BigDye Terminator v3.0 (Applied Biosystems, UK) and sequenced following ethanol precipitation (ABC) or G-50 Sephadex protocol (MotN; GE Healthcare, USA) according to manufacturer's instructions. To investigate sequence fidelity and DNA damage, four ancient sequences, representing the temporal range of specimens, were cloned using the Invitrogen (UK Ltd.) Topo-

TA bacterial cloning kit according to manufacturers instructions (Appendix 1). From each PCR product, 10 bacterial colonies were selected for sequencing (as above) and compared with the previously recovered direct sequencing. Finally, extraction and amplification of seven ancient caribou samples was independently replicated at the ABC and MotN following the different protocols described above for each laboratory.

2.4. Data analysis

Modern and ancient mitochondrial DNA sequences were aligned manually in Se-Al v2.0a11 (Rambaut 1996). We first created a descriptive median-joining network using Network v.4.5 (Bandelt *et al.* 1999; Fluxus Engineering 2008). We ran a Maximum Parsimony post-processing step (Polzin & Daneschmand 2003), and down-weighted the highly variable mutational positions 183, 217, 268, 315 and 444 by 50%, as suggested in the NETWORK user's guide. To identify maximally differentiated, but geographically homogenous population structure without conditioning the analysis on *a priori* population structures (groupings of 1 or more populations), we employed the program Spatial Analysis of MOlecular Variance, SAMOVA v.1.0, (Dupanloup *et al.* 2002; http://web.unife.it/progetti/genetica/Isabelle/samova.html). The SAMOVA analysis was run using 1,000 initial states and geographic groupings from K = 2 through K = 6. We selected K by identifying a plateau in the decrease of the total genetic variance that could be explained by differences among populations within groups. *Alligator* and *Sandpiper* ice patches (Figure 1, Appendix 1) contain ancient samples spanning a large time period (2,000 years and ~5,500 years,

respectively) and have bimodal age distributions. We therefore ran the SAMOVA analysis twice: first considering them a single 'population' and second with the two patches each subdivided into 'recent' and 'ancient' samples (recent ice patch samples are denoted with an "R", e.g. SandpiperR). For both populations, recent samples represent those <1,000 yrsBP, with ancient samples >>1,000 yrsBP as identified by the bimodal age distribution. Because each population within SAMOVA must have a unique geographic location, the coordinates of recent samples from Alligator and Sandpiper ice patches were shifted less than 0.05° north and west of the locations used for ancient Alligator and Sandpiper ice patch samples.

Although SAMOVA is not preconditioned on population structure (predefined groups of populations), individual samples must be placed within individual populations *a priori*. Defining these populations *a priori* and not explicitly incorporating information about variation in population range locations through time, sample age or evolutionary history may influence the optimal SAMOVA groupings. To properly incorporate these factors into our investigation, we utilized Bayesian phylogenetic reconstruction approach using the Bayesian genealogical inference package BEAST v.1.4.8 (Drummond & Rambaut 2007). A major advantage of BEAST is that it allows the explicit incorporation of non-contemporaneous DNA sequences in a coalescent-based inference framework. Each BEAST analysis was run for 30,000,000 iterations, sampling every 3,000, with the first 3,000,000 steps discarded as burn-in. We selected the optimal evolutionary model using the software ModelGenerator v0.85 (available from

bioinf.may.ie/software/modelgenerator/). For all BEAST analyses we used an HKY model of nucleotide substitution with gamma-distributed rate heterogeneity (Hasegawa *et al.* 1985) assuming a strict molecular clock and a constant population size. We also tested a more generalized model allowing for dynamic population changes (the Bayesian Skyline plot; Drummond et al. 2005), however Bayes Factor analysis (Newton & Raftery 1994; Suchard et al. 2005) showed stronger support for the simpler constant population size model (Bayes Factor for Constant Population size vs. Bayesian Skyline Plot = 2.10; in log10 units where a value of greater than 2 indicates strong support for hypothesis one over hypothesis two). Estimated sample sizes (ESS) for each parameter in each independent chain were examined using Tracer v. 1.4. (Rambaut & Drummond 2007) to evaluate mixing, and plots of posterior samples were evaluated by eye to ensure each run reached stationarity.

2.5. Results

2.5.1. Sequence authenticity

Consistent with the level of preservation of the ancient remains used in this study, we observed no evidence of contamination or DNA damage. Cloned reagent and PCR negative controls produced no recoverable DNA. The four extracts selected for bacterial cloning produced identical sequences to those previously recovered from direct sequencing of these same extracts. Finally, each of the seven sequences that were extracted and sequenced at independent facilities, using different extraction and amplification protocols, resulted in identical sequences (Kuhn et al. 2010). This exceptional preservation is further

demonstrated by recovery of STR loci from several ancient samples (TSK, PG and BS, unpublished data; J. Haile, personal communication). However, due to the limitations of the available ancient samples, detailed STR analysis of these ancient samples could not be performed.

2.5.2. Modern plus ancient Mitochondrial DNA Analyses

All ancient and modern haplotypes included herein are representatives of the Beringian-Eurasian Lineage (as defined by Dueck 1998; Flagstad & Røed 2003; and McDevitt *et al.* 2009; analysis and results not shown). As a result, we felt it inappropriate to include many previously published modern haplotypes that are representatives of the North American Lineage as the very large genetic distance between these two groups would likely have diminished our ability to assess regional phylogeographic patterns.

The median-joining network shows considerable sequence diversity within the caribou mtDNA control region (Figure 2). Two high frequency haplotypes are centrally located within the network (Figure 2; haplotypes Rt003 and Rt007), and correspond to the only two haplotypes observed in the Aishihik herd. The haplotypes found in the Carcross and Ibex herds tend to be on short branches related to these two common sequences. In contrast, haplotypes observed in the larger Fortymile herd (haplotypes in blue in Figure 2) are distributed throughout the network, but tend to be on longer branches arising from the central portion of the network. Ancient haplotypes show two similar patterns: short branches generally arising from a central ancient haplotype, or long branches similar to the Fortymile haplotypes. The network identifies one small grouping of haplotypes

(Clade 1, Figure 2) that appear closely related, with the remaining haplotypes forming a diverse cluster with no clear internal structures (Figure 2). Clade 1 is composed of the dominant haplotypes from two of the Southern Lakes herds (Ibex and Carcross), one of the two haplotypes observed in the Aishihik herd and several ancient haplotypes, all more recent than ~800yrsBP. Clade 1 also contains a number of haplotypes found only in the Fortymile herd. The herds that represent haplotypes identified within Clade 1 correspond to those herds indentified within Cluster 1 of the STR analysis (Kuhn et al. 2010), but with one key difference: Clade 1 also includes several Aishihik and Fortymile herd haplotypes. For this reason, we distinguish Clade 1 as representing *mtDNA* genetic structure and Cluster 1 as representing the corresponding *STR* genetic structure.

The mtDNA SAMOVA results for the multiple K values examined for mtDNA data are summarized in Table 2. These results suggest that three clades (K = 3) account for the optimal genetic/geographic structure, accounting for 25.5% of variation at the Among Groups level (Fct = 0.255, $p << 0.0001$). The three groups are: 1) Carcross, Ibex, *SandpiperR, Upper Jo Jo*; 2) Fortymile, Aishihik, Wolf Lake, and all Ancient samples excluding *SandpiperR* and *Upper Jo Jo*; and 3) *Thulsoo*. Ignoring the age dichotomies in *Alligator* and *Sandpiper* ice patches produced a similar but less optimal structure (K = 3, 22.6% of variation at the Among Groups level, Fct = 0.226, $p << 0.0001$). Geographic position was found to have no effect on the ability of SAMOVA to recover the optimal outcome (results

not shown). Excluding the *Thulsoo* sample from the analysis returned an optimal K value of K = 2, with the same clades described above (results not shown).

The Bayesian reconstructions, which were performed so as to incorporate explicitly the temporal component of the data, reveal a single well-supported clade (posterior support of 0.93; Figure 3) that includes the same haplotypes isolated in Clade 1 identified in the network analysis (Figure 2). The corresponding herds which contained these Clade 1 haplotypes mirror the SAMOVA grouping of the modern Ibex and Carcross herds, plus recent (<1000 yrsBP) *Sandpiper* and *Upper Jo Jo* ice patch samples.

2.6. Discussion

Our analyses reveal a dynamic recent history for caribou in southern Yukon. The rapidly evolving STR loci identify strong inter-herd genetic structure at broad geographic scales (Kuhn et al. 2010). More slowly evolving maternally inherited mtDNA supports some of these multi-herd genetic groupings (specifically a clade encompassing herds from the Southern Lakes region), and when coupled with the STR data, identify past genetic mixing and possible founder effects (specifically the lack of correlation between STR and mtDNA markers for the Aishihik herd). Finally, the addition of data from ancient caribou allow novel inference about the medium-term geographic and genetic stability of caribou populations in the southern Yukon.

We identify a lineage replacement occurring in the Southern Lakes region. Modern evidence (mtDNA and STR) differentiate the herds of the Southern

Lakes region (Atlin, Carcross and Ibex herds; Clade 1 and Group 1) from surrounding caribou herds. Interestingly, ancient ice patch caribou samples older than 1,000 yrsBP and recovered from this region do not group with this lineage, but instead are indistinguishable from the modern day surrounding herds, including the large migratory Fortymile herd. The evidence suggests that around 1,000 yrs BP the caribou occupying the Southern Lakes and found in associated ice patches suffered a population decline resulting from either displacement or extirpatition from the area. Following a return to suitable conditions, the region was recolonized by a different lineage. As this lineage is not sister to all other caribou, it suggests that this distinguishable lineage likely reflects recolonization by an adjacent northern mountain herd, or a small remnant population that survived the population decline in some local refugia.

This replacement event can be further clarified using dietary analysis of the ancient ice patch caribou. In a recent analysis of ancient caribou diet, Farnell et al (2004) analyzed fecal material collected at the *Friday Creek* ice patch, and found that ice patch caribou had a diet similar to the small sedentary northern mountain caribou herds that inhabit the Southern Lakes region today (Ibex, Carcross and Atlin herds; STR Cluster 1). This distinguishes these ancient caribou from the large migratory Fortymile herd whose historic range encompassed this ice patch as recently as 100 years ago (Boertje & Gardner 2000). This dietary analysis is consistent with modern caribou behaviour and habitat usage, where northern mountain caribou undergo modest altitudinal migrations from their winter range in valley bottoms to higher elevations during

the summer. At higher elevations, these caribou are known to aggregate on alpine ice patches to avoid insect harassment (Weladji *et al.* 2003). In contrast, the migratory Fortymile herd would have occupied its southern range limits during the winter months, when individuals are most dispersed, the ice patches are covered in deep winter snow and suitable habitat is only found in valley bottoms. In support of these divergent life histories, we find little evidence of gene flow between the Fortymile herd and the herds of the Southern Lakes region. These results are in contrast to observations from Alaska, where an expanding migratory herd introgressed with the resident sedentary herds to such an extent that individual herd identification became impossible (Hinkes *et al.* 2005). However, significant connectivity between migratory and northern mountain herds appears to exist for the herds surrounding the Southern Lakes region (Bonnet Plume, Finlayson, Klaza and Tay River herds; members of STR Cluster 3 in Kuhn et al. 2010). These herds, that are behaviourally similar to the Southern Lakes herds, are genetically indistinguishable, using fast evolving STRs, from the migratory Fortymile herd (Kuhn et al. 2010). This close genetic history between migratory and sedentary ecotypes suggests that significant gene flow still exists between these geographically dispersed herds; a result that is at odds with present management framework that treats these herds as separate managament units.

STR and mtDNA data do provide differing results with regards to the evolutionary relationships of several the sedentary herds. In the STR analysis (Kuhn et al. 2010), the Aishihik herd forms a distinct cluster (STR cluster 2),

suggesting strong isolation from surrounding herds. Had this herd been isolated from surrounding herds for several thousand years, we would have expected that Aishihik caribou and the ancient caribou recovered from ice patches within their geographic range (*Gladstone*, *Lower Gladstone* and *Thulsoo* ice patches) would form a distinct clade. However, all of the modern and ancient mtDNA haplotypes from this herd are shared with the surrounding herds. Unfortunately, we were unable to isolate useable STRs from these ice patch remains. The distinctiveness of the Aishihik herd observed using STR markers is then best explained as the result of recent founder effects or population bottleneck coupled with short-term isolation. Population estimates for the Aishihik caribou herd document a 50% reduction in herd size between 1981 and 1993, which precipitated an extensive predator control and management strategy designed to stabilize this economically and culturally important herd (Hayes *et al.* 2003).

In contrast to the Aishihik herd, both STR and mtDNA results provide evidence for the distinctiveness of the Ibex, Carcross and Atlin herds of the Southern Lakes region (mtDNA Clade 1; STR Cluster 1). Had these herds been isolated from others for the last several thousand years, caribou recovered from *Alligator, Bratnober, Friday Creek, Texas Gulch, Thandlät, Sandpiper* and *Vand Creek* ice patches should have fallen within this group. However and as stated previously, all but one recent (<1,000 yrsBP) ice patch sample from the Southern Lakes region show close genetic affinity with the herds present on the landscape today, with our results identify a distinct transition occurring at ~ 1,000 yrsBP. Caribou samples older than this invariably fell outside of the well-supported

Clade 1, and instead were indistinguishable from modern Aishihik and Fortymile samples and ice patch samples from the Aishihik range.

While this proposed replacement event explains most of the differences in caribou genetic diversity observed before and after 1,000 yrsBP, there are several exceptions. For example, the small but diverse Ibex caribou herd contains mtDNA haplotypes found both within and outside Clade 1 (Figure 2). These haplotypes are not shared with any of the herds tested here, or any of the previously published sequences (Figure 2; nodes representing Ibex samples are not shared with other herds). Similarly, a small number of Fortymile haplotypes are found within Clade 1. These exceptions may represent recent dispersal and gene flow within the Southern Lakes region or ancestral haplotypes that survived the replacement event. Given this, we adopt a conservative interpretation of partial replacement to explain the 1,000 yrBP replacement event.

The timing of the proposed partial replacement event is consistent with two large-scale environmental disturbances within the southern Yukon: i) the eruption and deposition of the White River tephra, and ii) the global climate warming of the Medieval Warm Period (MWP).

The White River tephra was deposited in two lobes: the first, smaller, northern lobe between 1,900 – 1,500 yrsBP, and the much larger, eastern lobe ~ 1,200 yrsBP (Figure 1; Clague *et al.* 1995; Robinson 2001). The eastern lobe represents the largest known Holocene stratovolcano (Lerbekmo 2008), with an estimated 47 km^3 of volcanic ash deposited across approximately 540,000 km^2 (Lakeman et al. 2008; Robinson 2001), and ash thicknesses of up to 30cm

having been recorded more than 300km from the source volcano (Bostock 1952). Perhaps unsurprisingly, the deposition of this lobe has been linked to extensive archaeological upheaval in the southern Yukon (Hare et al. 2005; Moodie et al. 1992; Robinson 2001; Workman 1979).

The detrimental effects of ash fall on livestock and caribou have been observed from 2.5 – 10 cm of ash deposited from the 1912 Mount Katmai eruption in Alaska (Jagger 1945) and as little as 1.9 cm of ash following the 1947 eruption of Hekla, in Iceland (Malde 1964). Given that much of the Southern Lakes region falls within the 5cm isopach (ash depths of 5cm or greater) of the eastern lobe of the White River tephra (Figure 1), it seems likely that this cataclysmic event had a noticeable effect on the distribution of caribou populations living within the region at the time.

The appearance of Clade 1 in the Southern Lakes region at ~1,000 yrsBP follows a 400 year period during which no remains were preserved within the sampled ice patches (1440 to 1030 yrsBP, Farnell *et al.* 2004). This hiatus is coincident with the increase in global temperatures associated with the MWP (Farnell *et al.* 2004; Helama *et al.* 2009). An increase in mean July temperatures is also inferred from both pollen and chironomid accumulation rates within our study region (Bunbury & Gajewski 2009). Warmer temperatures mean more frequent periods of winter thawing, increased winter snowfall, increased insect harassment and loss of perennial snow patches (Thomas & Gray 2002; Weladji *et al.* 2003), all of which have detrimental effects on caribou. It is therefore possible, even likely, that the MWP also played a role in the extirpation of caribou

from the Southern Lakes region, with subsequent recolonization occurring as temperatures cooled during the Little Ice Age. Interestingly, and as a result of warmer summer temperatures observed in recent years within our study region, the vast majority of sampled ice patches, which had persisted for upwards of 7,000 years, no longer exist.

The combined analysis of ancient and modern caribou provides several insights for caribou management and conservation. First, combined STR and mtDNA analyses demonstrate that the Southern Lakes caribou herds are distinguishable from other caribou herds, but that recent shared ancestry or gene flow has blurred this genetic division on smaller spatial scales. Our results support management of these three herds (Ibex, Carcross and Ibex) as a single management unit, and provide genetic evidence in support of active management practices designed to ensure their long-term persistence.

Interestingly, however, the most distinctive herd based on STR data (Kuhn et al. 2010), the sedentary Aishihik herd, is indistinguishable from the adjacent migratory Fortymile herd when using mtDNA. However, a dichotomy between STR and mtDNA analysis is not unsurprising given observations for mountain caribou from the Central Rocky Mountains of British Columbia and Alberta (McDevitt *et al.* 2009). In contrast, where McDevitt et al. (2009) observed a dichotomy between markers for herds of the same ecotype with recent mixing of two divergent mitochondrial lineages, the dichotomy observed in southern Yukon is between different ecotypes, but where we see STR segregation within a single mitochondrial lineage. The unique genetic information contained within the

Aishihik herd is then a very recent phenomenon. Coupled with the observed genetic relationships between woodland herds of the central Yukon, we suggest that the total unique genetic diversity contained within these herds remains very small and of recent origin. If this is true, then the loss of any one herd would not have a significant effect on the overall genetic diversity of caribou within the region. It is however important to acknowledge the economic and cultural importance of each individual herd within its region. Our results underscore the importance of using multiple lines of evidence when making appropriate management decisions, as analysis of STR markers alone could have lead to differing management priorities regarding the Aishihik herd.

Finally, by adding a temporal perspective to our analysis, we identify a recent, partial replacement event that is not discernible from modern data alone. This replacement event was most likely caused by changes in caribou habitat as a result of either the MWP or the deposition of the White River tephra. At present we can not distinguish between these two closely timed events, however increased sampling and stratigraphic coverage of the 1,500 to 500 yrBP period may be sufficient to identify a single causal mechanism. Whether this replacement event was caused by a large volcanic eruption or increased snowfall and warmer temperatures, caribou were likely able to recolonize the large region in the southern Yukon as a result of their ability to expand in numbers and migrate into newly available habitats as cooler temperatures of Little Ice Age prevailed (Bunbury & Gajewski 2009; Viau et al. 2006).

In the face of current climate change, with temperature increases predicted to be an order of magnitude larger than that observed during the Holocene (Viau *et al.* 2006), preserving connectivity between isolated patches of caribou habitat may become more important than the individual preservation of small, isolated sedentary herds. With ongoing research into the recoverability and usability of STR markers from ancient samples, we hope to further test these hypotheses.

Primer	Sequence 5' to 3'	Temp (°C)
Rtp2f	TCTCCCTAAGACTCAAGGAAG	57
Rtp2r	GGCTATTGAGTGCAGAACTG	
Rtp3f	TCCACAAAATTCAAGAGCCTT	55
Rtp3r	TGGGGCATATAATTTAATGTAC	
RT1§	TAAACGTACATATATGGTCCTG	57
B- 16168H§	TGGTTTCACGCGGCATGGT	
CP1	GYCAACATGCGTATCCCG	52
CP2	RTGAGATGGCCCTGAAGAAA	
CB1F	TCAACACCCAAAGCTGAAGTT	55
CB1R	TGGCTATTGAGTGCAGAACTGT	
CB2F	CAAAATTCAAGAGCTTGTCAG	55
CB2R	TGTACTATARYCGTACARRACCA	
CB3F	CACTCAATAGCCATATTATATCTTAAA	55
CB3R	ACGATCAACAATATTATGTACTATG	
CB4F	AAATTATATGCCCCATGCTT	55
CB4R	GTTTCACGCGGCATGGTA	
CB5F	CCCCCTAGATCACGAGCTTA	55
CB5R	GTGGGCGATTTTAGGTGAGA	

Table 1.- Primer pairs. CB## primers used for extractions at MotN. § – modified from Gravlund et al. (1998).

K	Fst (%var)	Fsc (%var)	Fct (%var)	Structure
2	0.40515 (59.48)*	.21069 (15.88)*	0.24637 (24.64)†	(Thulsoo) (Remainder)
3	**0.34865 (65.13)***	**0.12612 (9.40)***	**0.25465 (25.46)***	(Thulsoo) (Carcross,Ibex,SandpiperR,Ujo) (Remainder)
4	0.34579 (65.42)*	0.10920 (8.02)*	0.26559 (26.56)*	(Thulsoo) (Carcross,Ibex,SandpiperR,Ujo) (Wolf,Irvine) (Remainder)
5	0.34419 (65.58)*	0.10023 (7.31)*	0.27113 (27.11)*	(Thulsoo) (Carcross,Ibex,SandpiperR,Ujo) (Wolf,Irvine) (Vand) (Remainder)
6	0.30317 (76.82)*	-0.10242 (-7.14)*	0.30317 (30.32)*	(Thulsoo,Gladstone,Bratneber)(Carcross,Ibex,SandpiperR,Ujo) (Wolf,Irvine) (Vand,Friday,Thandlat,AlligatorR,Texas) (Aishihik,AlligatorA,SandpiperA)(Fortymile)

Table 2.- Results from Spatial Analysis of MOlecular Variance for K = 2 – 6. *) p << 0.005; †) p=0.054. Optimal grouping is K = 3 (bold), as increased K values beyond K = 3 result in minimal increase to Fct. The analysis was also run excluding the Thulsoo ice patch sample (not shown), which produced similar results to those reported here and an optimal K of K = 2. SAMOVA consistently identifies the Southern Lakes caribou herds as distinct from surrounding herds at all K values.

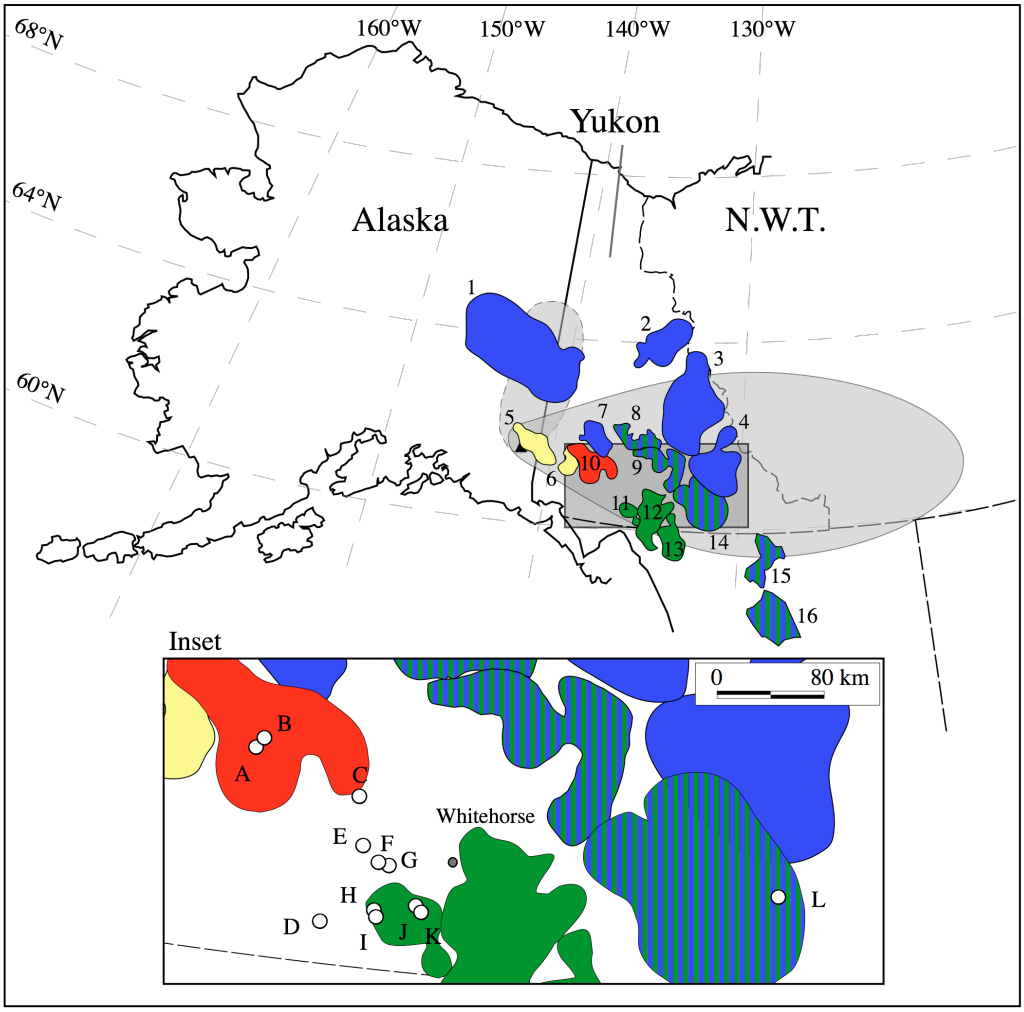

Figure 1.- Location map of Yukon and Alaskan caribou herds showing herd ranges estimated in 2008 (adapted from, Yukon Environment Geomatics 2008; Zittlau 2004). Only herds referred to in (Kuhn et al. 2010) or within the present study are shown: 1) Fortymile, 2) Bonnet Plume, 3) Tay River, 4) Finlayson, 5) Chisana, 6) Kluane, 7) Klaza, 8) Tatchun, 9) Pelly, 10) Aishihik, 11) Ibex, 12) Carcross, 13) Atlin, 14) Wolf Lake, 15) Cassiar and 16) Chase caribou herds. Colors represent region scale structure identified using 8 microsatellite loci in Kuhn *et al.* (2010): green) Cluster 1 – Southern Lakes caribou herds; red) Cluster 2 – Aishihik herd; blue) Cluster 3 – Fortymile, Pelly, Tay River, Wolf Lake herds; and yellow) Cluster 4 – Chisana and Kluane herds. Herds color coded with blue–green strips represent herds that were not assigned to a single cluster, but equally to either Cluster 1 or 3. Inset shows detailed view of ice patch locations for samples used in this study: A) *Gladstone (IP36)*, B) *Lower Gladstone (IP35)*, C) *East Thulsoo (IP50)*, D) *Vand Creek (IP34)*, E) *Bratnober (IP18)*, F) *Upper Jo Jo (IP31)*, G) *Thandlät (IP1)*, H) *Texas Gulch (IP52)*, I) *Sandpiper (IP29)*, J) *Friday Creek (IP37)*, K) *Alligator (IP38)*, L) *Irvine (IP80)*. The solid line, grayed area represents the observed extent of the eastern lobe of the White River tephra laid down during the 1,200 yrsBP eruption. The dashed, grayed area represents the observed extent of the smaller northern lobe, deposited between 1,900 – 1,500 yrsBP. Data modified from Robinson (2001).

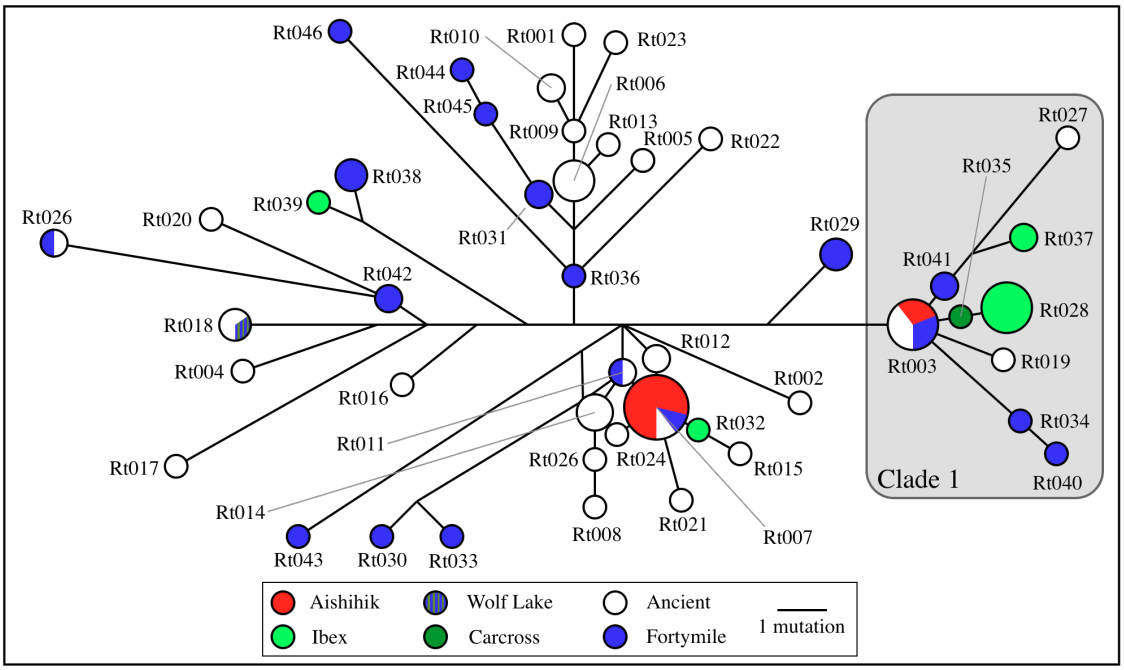

Figure 2.- Median-joining network of all ancient and modern haplotypes. Appendix 1 provides further information on sampling locations, ages and haplotype frequencies (haplotype labels identify unique haplotypes only). Colors match classification from Figure 1, with the herds of Cluster 1 (green) divided into shades, see legend. Clade 1, outlined in grey, is the only visually distinct group within the diverse network, and its member haplotypes correspond closely with haplotypes found in populations identified using SAMOVA (Table 2), and results from BEAST (Figure 3).

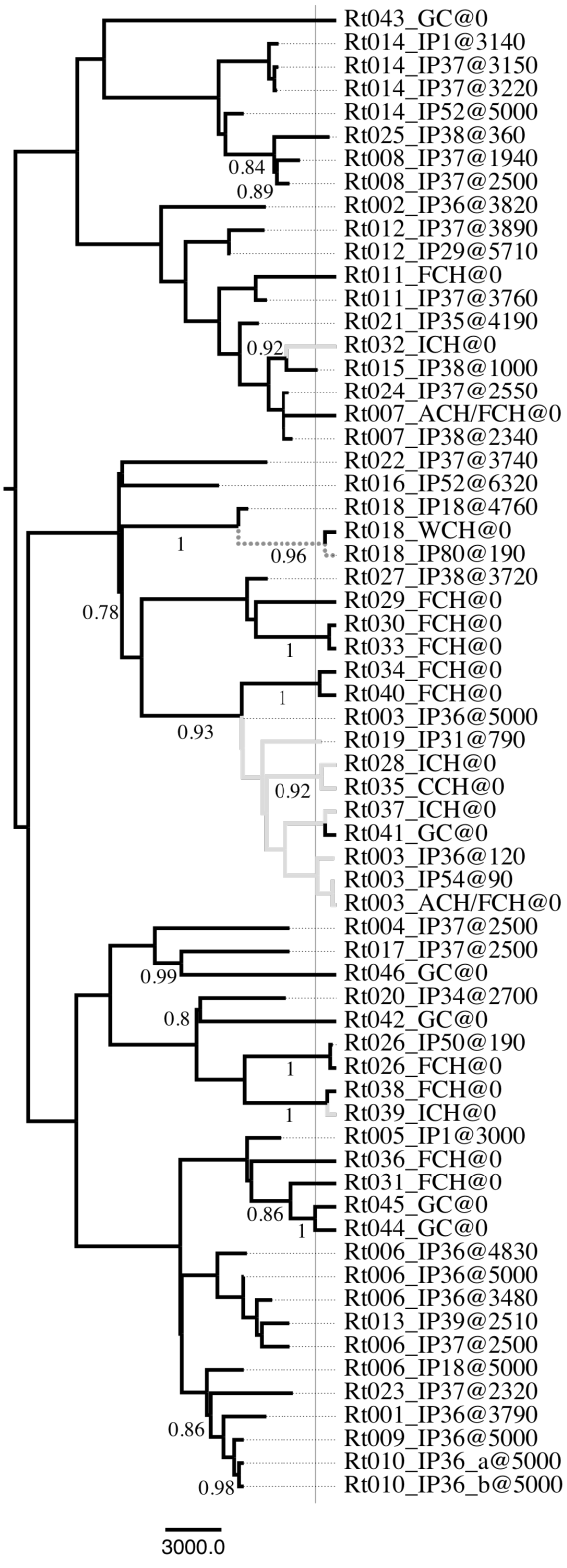

Figure 3.- Maximum Clade Credibility genealogy summarized from the BEAST analysis described in the main text. Posterior support values greater than 0.75 are shown adjacent to each node. Line styles indicate structure obtained from Spatial Analysis of MOlecular Variance (Table 2). Branch lengths are measured in radiocarbon years before present, with the rate estimated from dated sequences. The only well-supported clade comprises the same samples that are identified as Clade 1 in the median-joining network. Similar to the tree topology, the optimal SAMOVA structure includes the Southern Lakes herds as a separate group (mtDNA Clade 1), however, as the SAMOVA approach requires samples to be assigned to an individual population *a priori*, several individual haplotypes appear to represent recent migrants to or from the Southern Lakes mtDNA Clade 1. All ancient samples dated before ~1,000 yrsBP (indicated by the thin gray vertical line) group outside of the well-supported Clade 1, indicating that ancient caribou from the Southern Lakes region do not represent the ancestral source of present day Southern Lakes herds.

CHAPTER 3. ANCIENT INSIGHTS INTO CONSERVATION OF SAIGA ANTELOPE

Conservation biologists understand that linking demographic histories of species at risk with causal biotic and abiotic events should help us predict the effects of ongoing biotic and abiotic change. In parallel, researchers have started to use ancient genetic information (aDNA) to explore the demographic histories of a number of species present in the Pleistocene fossil record. However, aDNA studies have primarily focused on identifying long term population trends, linked to climate variability and the role of early human activity. Population trends over more recent time, e.g. during the Holocene, have been poorly explored, partly due to analytical limitations. In the recent investigation into the demographic history of the compelling and critically endangered saiga antellope (*Saiga tatarica*), Campos *et al.* (2010a) highlight the potential of aDNA to investigate demographic patterns over more recent time periods. The time may come when past and current demography can be combined to produce a seamless record.

In order to investigate these recent demographic patterns, Campos et al. utilized Approximate Bayesian Computation (Beaumont *et al.* 2002) to distinguish between competing biologically-relevant models simulated in Bayesian Serial SimCoal (BayeSSC; Anderson et al. 2005; Ramakrishnan & Hadly 2009; as implemented at http://www.stanford.edu/group/hadlylab/ssc/).

BayeSSC was introduced as an alternative parameter estimation approach to the widely used Markov Chain Monte Carlo (MCMC) search algorithm applied to serial coalescent processes (BEAST; Drummond & Rambaut 2007). Within the coalescent-based BEAST framework, Bayesian MCMC is used to estimate a suite of evolutionary and demogrpahic parameters, without requiring that the underlying "true" topology be known. However, within this coalescent framework, demographic parameters are limited to a variety of simple single population models (e.g., constant population size, exponential growth, and the explanatory Bayesian Skyline) for which the coalescent likelihood function can be defined (Ramakrishnan & Hadly 2009). Unlike BEAST, BayeSSC is not limited to demographic models where the likelihood function can be defined. It is constructed from the serial coalescent simulation platform SSC (Anderson et al. 2005; itself based on the widely used SimCoal 1.0 simulation program). Through the application of Approximate Bayesian Computation (ABC) to these simulated datasets, parameters estimated from competing modeled simulations can be compared to the parameters of the actual dataset. In this way, researchers are freed from the stringent requirements of simple single population models. In addition, researchers can now test specific models, rather than relying on a simple visual assessment of the patterns observed within a Bayesian Skyline plot.

It should be mentioned that this approach, although providing far more flexibility in implementation of a demographic model, does suffer from the problem of being restricted in the possible models it considers. BayeSSC can

effectively select the “optimal” model from a finite input suite of implemented models (e.g., in the case of saiga, Campos *et al.* implemented four competing models), but provides no method of identifying an underlying “true” model from the universe of possible histories. Nor is there a method of estimating how close the model is to the “true” history, unlike the MCMC parameter estimation approach which provides some level of certainty based on the likelihood framework.

Acknowledging these limitations, the BayeSSC approach can still provide valuable insights, in particular where structured populations are considered likely, or where the genetic variability (e.g., sample size, sequence variation, number of loci) do not provide sufficient information for MCMC demographic estimation. Finally, if the Bayesian, or approximate Bayesian, methodology is to be followed, the central tenant of Bayesian statistics that knowledge of an expert observer should contribute to the analytical results must hold true. In other words, it is difficult to dismiss the suite of models chosen as the most biologically plausible models solely due to an inability to test all theoretically possible models.

By making use of the BayeSSC approach, Campos *et al.* were able to identify a dramatic (on the order of 70%) recent population decline. This decline was attributed to either a sudden population bottleneck or geographic fragmentation followed by the loss of a distinct population. Even with the limited genetic information recoverable from ancient saiga remains (the authors discuss some of the challenges with aDNA extraction, challenges that certainly ring true), Campos *et al.* were able to determine that, under the population bottleneck

model, the decline was sudden and occurred some time prior to 2,000 yrs BP. Under the geographic fragmentation model, the lineage extinction appears to be contemporaneous with major climatic changes at the Pleistocene/Holocene boundary.

3.1. Past versus present declines.-

This dramatic population decline becomes a particularly interesting and disconcerting pattern when we consider more recent population trends within saiga. Although this fecund and highly mobile species has demonstrated an impressive ability to take advantage of good times, as illustrated by their colonization of North America in the late Pleistocene (Harrington & Cinq-Mars 1995), they have equally demonstrated their extreme susceptibility to bad times. Campos *et al.* discuss the near decimation of this species following the dissolution of the former Soviet Union, when the global saiga population dropped by an estimated 95%. The full record of demographic trends for saiga reveals a series of dramatic population declines linked to various abiotic and biotic factors. Beginning in 1970s, there were two dramatic and sudden population declines resulting from *dzhuts* – severe winters when surface ice crusts formed or deep snow accumulated. During the winter of 1971-72, approximately 400,000 saiga corpses were counted in an area of 36,000 km^2 (Bekenov *et al.* 1998). Four years later, another *dzhut* caused the deaths of a further 100,000 animals (Bekenov *et al.* 1998). This pattern of extreme winter mortality was observed again in 1987-88 and 1993-1994. Unfortunately, recent dramatic population declines have also been linked to biotic factors such as disease. In 1967, an

outbreak of foot-in-mouth disease in Kazakstan caused the deaths of an estimated 50,000 calves (Fadeev & Sludskii 1982). In the 1980s, two outbreaks of *pasteurellosis* caused the deaths of more than 170,000 saiga in Kazakhstan (Fadeev 1986; Fadeev & Sludskii 1982). And most recently in Kazakhstan, nearly 50% of the largest remaining saiga population died suddenly (Saiga Conservation Alliance 2010).

Within the context of applying aDNA to conservation issues, population declines such as those observed for saiga have been considered sufficient to obscure deeper (older) demographic patterns (Leonard 2008). However, this assertion has yet to be fully tested. Within the context of population declines observed for saiga, we make use of a simple simulation scenario to test how fluctuations over short time periods near the present affect the signal of past demographic changes. A strong effect would justify the use of aDNA to improve the demographic inference for this species.

In order to test both the effect of a modern decline on inferred demographic history, and thus the relevance of ancient DNA datasets to species of population concern, we make use of a hybrid simulation approach where datasets are simulated in SSC and analyzed using a Bayesian Skyline Plot (BSP; Drummond et al. 2005). This type of analysis has been done to a limited extent in published literature to test the effects of paleontological sampling strategies on inferred demographic histories (see, e.g., suppl. materials in Campos et al. 2010b). Here I simulate multiple datasets, mimicking multiple unlinked loci with the same demographic history/evolutionary parameters, to investigate the

sensitivity of BSP reconstructions. Although I focus on scenarios that shed light on the importance of ancient DNA samples, it is worth noting that this hybrid approach could provide the basis for a more statistical approach for interpreting BSPs through methods similar to wiggle-matching (Christen & Litton 1995).

3.2. Methods.-

Using Serial SimCoal (SSC; Anderson et al. 2005), we simulated a demographic history with sudden population declines at 100 yrsBP and 10,000 yrsBP. We simulated one suite of datasets with 50 modern taxa, and 50 ancient taxa (10 taxa every 10,000 years starting at 5,000 yrsBP), as well as one suite of datasets with 100 modern taxa and no ancient sequences. In addition to these 2-bottleneck scenarios, we simulated another suite of datasets with a single bottleneck (at 10,000 yrsBP) scenario using only modern samples. For each of these sampling strategies we simulated a 1,000 bp fragment under a simple mutation model (no rate heterogeneity or invariant sites, equal base frequencies, mutation rate = 1E-7 mutations/site/year, and a transition/transversion ratio of 0.33), a starting (present day) effective population size of 5,000 and 80% population declines at each bottleneck event. The modern effective population size for the single bottleneck scenario was set at 25,000 to mimic the population size change of the older bottleneck in the 2-bottleneck scenario. In addition, we tested a more dramatic modern population decline (90%), which produced similar results to the 80% decline, and is not reported here.

To determine if the true demographic history was recovered we produced Bayesian Skyline plots (BSP; Drummond et al. 2005) for each of these scenarios.

We used the BSP approach because the true demographic history represents a single population of fluctuating size and the simulated datasets should provide sufficient information to recover the demographic history. In addition, it would be inappropriate to test for the presence of bottlenecks within a dataset simulated in SSC, using the BayeSSC approach based on the same simulation engine. Bayesian Skyline Plots were produced using BEAST outputs (Drummond & Rambaut 2007) in the program Tracer (Rambaut & Drummond 2007). Each BEAST analysis was run for 30,000,000 steps, sampling every 3,000, and discarding the first 10% as a burnin period, using the Bayesian Skyline Plot (10 bins) demographic model. For modern datasets with no calibration information, a strong normal prior was placed on mutation rate such that the posterior distribution of the mutation rate mimics that of the modern+ancient calibrated dataset. ESS values above 100 for all parameters were considered necessary to ensure adequate mixing, and plots of posterior samples were evaluated by eye to ensure each run reached stationarity.

3.3. Results.-

Examining first the results from the 2-bottleneck – ancient sample scenario, we see that the BSP did a reasonable job of reproducing the true demographic history (Figure 4; top). In all of the 10 replicates shown in Figure 4, a two stage population decline is recovered. The timing of the older decline recovered using BSP varies between 5,000 yrsBP and 15,000 yrsBP. In addition, many of the bottleneck events appear to be quite sudden. In contrast, for the 2 bottleneck – modern sample scenario approach, there is no evidence of

a two stage population decline (Figure 4; middle), although the onset of population decline generally starts around 10,000 yrsBP. It is clear then that the addition of ancient information does increase our ability to identify ancient bottlenecks within a simulated dataset, however, it is not clear from these two graphs that the modern only sampling approach fails to identify the older decline due to the obscuring effect of the modern population decline. When we consider the 1-bottleneck – modern sample scenario, there is evidence for a population decline beginning around 10,000 yrsBP and plateauing before the present (Figure 4; bottom). These results are however more variable, with a consistent underestimation of the ancient population size. It is important to note the difference between the 2 bottleneck –modern sampling scenario and the 1 bottleneck – modern sampling scenario. From visual comparison of these two graphs it does appear that although limited, the modern data alone can reproduce a 10,000 year bottleneck, and that a dramatic recent bottleneck will obscure this demographic history in modern data

3.4. Discussion.-

These simple simulations support the belief that recovery of demographic histories for species of conservation concern, i.e. those that are likely to have undergone recent and dramatic population declines, stand to benefit from the addition of ancient genetic material. However, within the context of the saiga antelope, the limited fragment length and limited sample size necessitate an approach that is more sensitive than the Bayesian Skyline Plot employed above.

As argued by Leonard (2008) and Ramakrishnan & Hadly (2009), and demonstrated here by Campos *et al.* (2010a), even limited aDNA data (few, short samples) can be combined with modern simulation techniques to get demographic insight into older events. In addition, Campos *et al.* bring the deep past and recent past closer together by estimating parameters from the early Holocene. It remains to be seen whether these two scales can be brought together, such that uninterrupted histories can be generated from aDNA and modern DNA collected across a finer time series (see, e.g. Figure 5).

Though Campos *et al.* (2010a) do not apply a conservation perspective to their interpretations, such a perspective is possible. Saiga have previously been considered an important indicator of dry steppe-like habitats (Harrington & Cinq-Mars 1995). The sudden population decline contemporaneous with the warming of the climate around the Pleistocene/Holocene transition adds increased support for the status of saiga as an indicator species with narrow habitat/climate tolerances. In the face of current anthropogenic pressures and recent climate change, this vulnerability may become important as suitable habitat shrinks. Another important implication of Campos *et al.*'s results relates to the speed of the decline. The simulation studies are consistent with population declines being effectively geologically instantaneous. This suggests that the more recent sudden population declines are not necessarily out of the ordinary for this species. In turn, this suggests that this species has been successful in the past not because it is resistant to population declines but because it can rebound from dramatic declines (Bekenov *et al.* 1998; Harrington & Cinq-Mars 1995). From the modern

conservation perspective, conservation effort may be better spent on activities which enable population expansion than on attempting to mitigate unpredictable population declines. Under this scenario, habitat and migratory corridors would seem to be the most important ingredients for saiga survival.

Understanding the recent population trends and their possible causal factors is not only of scientific interest, but provides a crucial foundation for conservation decisions. Inferences from ancient genetic information may deepen and so bolster these foundations significantly if its ability to identify cryptic demographic patterns can be reproduced for other species of conservation concern.

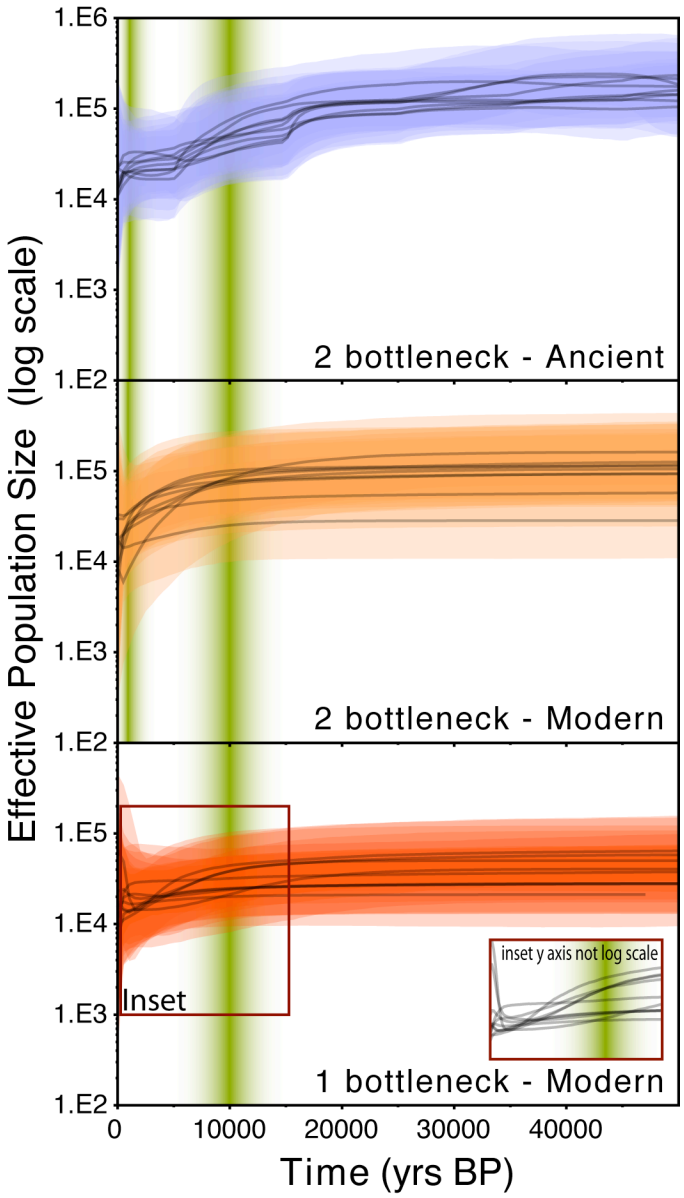

Figure 4.- Bayesian skyline plots showing the effect of a recent (100 yr) population bottleneck on inferred demographic history. In each panel, 10 simulations are shown with median and 95% confidence intervals. Simulated bottleneck events are highlighted in green. In the scenario with bottlenecks at 100 and 10,000 yrsBP and a 50/50 modern ancient sampling strategy, the BSP does well recovering both population declines (purple). In contrast, when only 100 modern samples are used, the 10,000 year bottleneck event is not observed (orange). When the modern bottleneck is removed, and only modern samples (n=100) are simulated, BEAST recovers a slight population decline (note the drop is compressed due to the log scale, see inset showing median estimates of Ne, not log(Ne)). Although these modern data due suggest a gradual population decline beginning around 10,000 yrsBP and plateauing before the present, the modern samples alone are not sufficient to recover the level of detail in the demographic history that is recovered when ancient sequences are used.

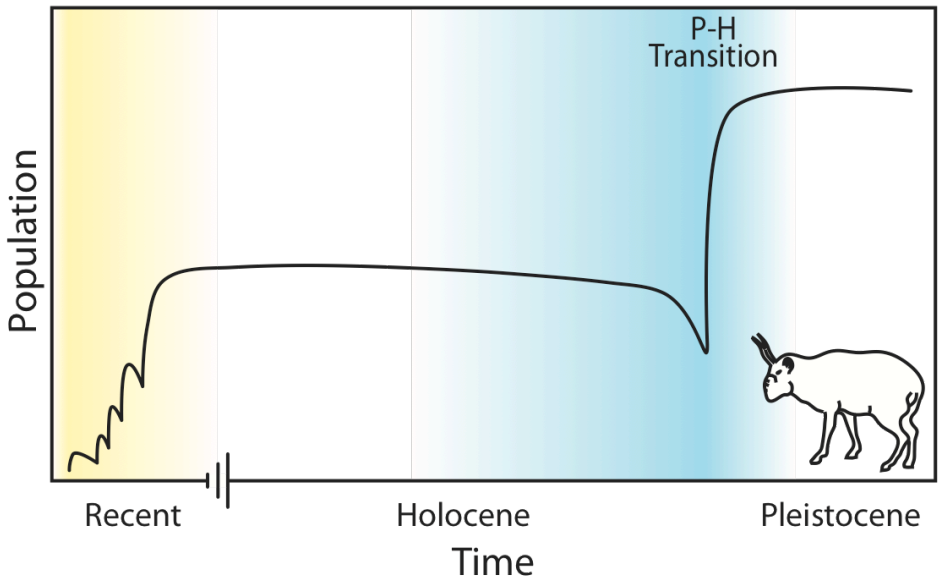

Figure 5.- Cartoon representing the possible demographic history of saiga inferred from Campos *et al.* (2010a) and recent documented population declines. The recent time (area in yellow) is drawn at an expanded scale to distinguish the multiple sudden dramatic population declines observed within the last 50 years. The earlier ~70% decline identified by Campos *et al.* is depicted at the paleontologically most likely time, the Pleistocene-Holocene (P-H) transition. Uncertainty in the actual date of this event is represented by the blue shading. We include hypothesized rapid population increases following these documented declines, in part due to the acknowledged high fecundity and mobility of saiga. It remains unlikely that the saiga population recovered to its Pleistocene high – a time when the saiga range extended as far east as the Yukon and Northwest Territories, Canada.

CHAPTER 4. CONCLUSION

It is perhaps not surprising that ancient genetic analysis of both saiga antelope and caribou describe dynamic fluctuating populations, to which application of a static management paradigm may well be inappropriate. For the saiga antelope, we describe the conservation implications of a recent study of Pleistocene and Holocene demographic patterns using ancient DNA (Campos et al. 2010a). This study identified two possible scenarios to explain modern and ancient genetic patterns. The first involved a two population model with recent disappearance of the largest population likely linked to the climatic transition from glacial to interglacial at the Pleistocene-Holocene boundary (~11,000 yrBP). The other possible model, a dramatic population bottleneck, is not associated with any major climatic transition, but again demonstrates the natural variability that may be inherent to the life history of this nomadic migratory species. In both cases, it is clear that the population decline was sudden and dramatic, findings which are in close agreement with the modern demographic changes observed for this species. From a management perspective, maintaining a stable population is not likely to be feasible. It will likely be better to explore management and conservation strategies that facilitate population recovery, and range expansion during fair weather.

Interestingly, I make a similar recommendation in a very different situation, with a similar northern species, caribou. In this case, rather than

investigating demographic changes, I employed ancient DNA recovered from mid to late Holocene alpine ice patches to assess the herd relationships between adjacent caribou herds in the southern Yukon, Canada. Through the combination of modern and ancient genetic information, I identified a unique clade comprised of members of the Southern Lakes caribou herds. This distinct clade was observed using both mitochondrial and microsatellite loci. More interestingly, from a temporal rather than geographic perspective, this unique clade represented a recent phenomenon, having appeared within the last ~1,000 years. Prior to that time, caribou present within the Southern Lakes region were more closely related to the surrounding genetically diverse herds.

This genetic replacement event coincided closely with two dramatic landscape disturbances, a major volcanic eruption and deposition of the White River tephra, and the climatic warming during the Medieaval Warm Period. Our information could not separate the two closely timed events, but the implications for management remain the same. In either case, the landscape was rendered less hospitable to caribou, causing the displacement or local extirpation of a very large ancient herd, with subsequent recolonization of the area when the situation improved. This recolonization was achieved by expansion of another herd into the unoccuppied territory. It is clear then, that the disappearance of caribou from a particular area may only be a serious consideration if there is a loss in the ability of surrounding herds to expand during favourable times. It should be mentioned that this inference is predicated on the return to favourable conditions

within a timely fashion. In the face of modern anthropogenic climate shifts, the loss of caribou from a region may be considerably more serious.

For both of these iconic and threatened arctic species, management practices must incorporate and foster population connectivity and the ability for population expansion. In our increasingly fragmented and anthropogenically controlled landscape, this may well pose a considerable challenge to management efforts, in particular in light of the compounding effect of anthropogenic climate warming. It may be worth noting that both the species considered in this thesis represent isolated bits of the mammal tree – they are, in other words, evolutionarily non-redundant (Isaac et al. 2007; Redding & Mooers 2006). It is well-documented that such very evolutionarily isolated species may be at greater than expected risk of extinction (see Appendix 2 and references therein). This should be added impetus for their ongoing conservation.

Identification of this dynamic history for caribou and saiga was only possible due to the additional information provided by ancient DNA sequences. It stands to reason that, in modern studies of phylogeography, there is potential to erroneously infer historical events when true events leave no signature within the modern phylogenetic distribution, as in the case of caribou herd replacement, or modern demographic changes mask older events, as in the case of saiga. The former of these scenarios has previously been identified in other species, including very recent loss of a unique lineage of bottlenose dolphins and hidden inter-population migration related to shifting ice dynamics in penguins (Nichols et al. 2007; Shepherd et al. 2005). Acknowledging that ancient DNA remains can

not be obtained for the majority of species, modern phylogeographers should consider the potential biases resulting from sampling of a single time period.

APPENDICES

Appendix 1: Caribou sampling information

Sample information. ‡ – Sample that was used in bacterial cloning authentication step. † – Sample used for independant extractions. § – Age estimated from distribution of 14C dates in same ice patch (G. Hare, personal communications, August 2007).

haplotype ID	Extraction ID	YHB ID	PCDNAR ID	Locality / Herd	Sample Type	GenBank Number	Haplotype Frequency	14C Date (yrBP)	Date Reference
	TK106		1.832	Ibex caribou herd	PCDNAR extract			0	
	TK108		ET282	Ibex caribou herd	PCDNAR extract			0	
	TK111		ICH/95F-B26	Ibex caribou herd	PCDNAR extract			0	
	TK113		ICH/95F-B48	Ibex caribou herd	PCDNAR extract			0	
Rt007_ACH/FCH@0	TK32		10351†	Aishihik caribou herd	fecal sample	GU327584	11	0	
	TK33		10348†	Aishihik caribou herd	fecal sample			0	
	TK71		ACH/95F-B3	Aishihik caribou herd	PCDNAR extract			0	
	TK73		ACH/95F-B73	Aishihik caribou herd	PCDNAR extract			0	
	TK74		ACH/95F-B82	Aishihik caribou herd	PCDNAR extract			0	
	TK75		ACH/95F-B84	Aishihik caribou herd	PCDNAR extract			0	
	TK76		ACH/95F-F1	Aishihik caribou herd	PCDNAR extract			0	
	TK77		ACH/95F-F3	Aishihik caribou herd	PCDNAR extract			0	
	TK79		ACH/96F-Y19	Aishihik caribou herd	PCDNAR extract			0	
	TK80		ACH/96F-Y34	Aishihik caribou herd	PCDNAR extract			0	
	TK102		105215	Fortymile caribou herd	PCDNAR extract			0	
Rt011_FCH@0	TK97		104824	Fortymile caribou herd	PCDNAR extract	GU327585	1	0	
Rt029_FCH@0	TK85		104811	Fortymile caribou herd	PCDNAR extract	GU327586	3	0	
	TK95		104822	Fortymile caribou herd	PCDNAR extract			0	
	TK100		104831	Fortymile caribou herd	PCDNAR extract			0	
Rt030_FCH@0	TK92		104819	Fortymile caribou herd	PCDNAR extract	GU327587	1	0	
Rt031_FCH@0	TK86		104812	Fortymile caribou herd	PCDNAR extract	GU327588	2	0	
	TK101		104842	Fortymile caribou herd	PCDNAR extract			0	
Rt032_ICH@0	TK107		1.87	Ibex caribou herd	PCDNAR extract	GU327589	1	0	
Rt033_FCH@0	TK87		104813	Fortymile caribou herd	PCDNAR extract	GU327590	1	0	
Rt034_FCH@0	TK94		104821	Fortymile caribou herd	PCDNAR extract	GU327591	1	0	
Rt035_ICH@0	TK81		CCH/95F-B51	Carcross caribou herd	PCDNAR extract	GU327592	1	0	
Rt036_FCH@0	TK88		104814	Fortymile caribou herd	PCDNAR extract	GU327593	1	0	
Rt037_ICH@0	TK109		ET284	Ibex caribou herd	PCDNAR extract	GU327594	2	0	
	TK110		ICH/95F-B19	Ibex caribou herd	PCDNAR extract			0	
Rt026_FCH@0	TK89		104815	Fortymile caribou herd	PCDNAR extract	GU327595	1	0	
Rt038_FCH@0	TK90		104816	Fortymile caribou herd	PCDNAR extract	GU327596	3	0	
	TK96		104823	Fortymile caribou herd	PCDNAR extract			0	

haplotype ID	Extraction ID	YHB ID	PCDNAR ID	Locality / Herd	Sample Type	GenBank Number	Haplotype Frequency	14C Date (yrBP)	Date Reference
	TK103		105216	Fortymile caribou herd	PCDNAR extract			0	
Rt039_ICH@0	TK104		0.671	Ibex caribou herd	PCDNAR extract	GU327597	1	0	
Rt040_FCH@0	TK84		104810	Fortymile caribou herd	PCDNAR extract	GU327598	1	0	
Rt041_GC@0				Grant's caribou	GenBank	AY178676	2	0	
Rt042_GC@0				Grant's caribou	GenBank	AY176678	2	0	
Rt043_GC@0				Grant's caribou	GenBank	AY178715	1	0	
Rt044_GC@0				Grant's caribou	GenBank	AY176716	1	0	
Rt045_GC@0				Grant's caribou	GenBank	AY176717	1	0	
Rt046_GC@0				Grant's caribou	GenBank	AY178720	1	0	

Appendix 2: Projects completed during my M.Sc., but unrelated to caribou aDNA research.

Summary of other work completed by TS Kuhn during thesis

Holocene Extinctions and the Loss of Feature Diversity

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Introduction.-

Species are hard to define, but, under all definitions they are unique. Each species can be considered to possess a set of unique characters which comprise its feature diversity - the diversity that would be lost when that species goes extinct. However, because species differentiate via evolution from other species, they also share features as a function of degree of relationship. So, within placental mammals, all mice share all the characteristics that allow us to call something a mouse, whereas the sole living species of aardvark shares very few basic mammal characteristics with anything: it must be true that the earth loses less diversity when any single mouse species goes extinct than when the last

living aardvark shuffles off its mortal coil. This is one well-established way in which species are not created equal (Vane-Wright et al., 1991; Faith, 1992).

We can start to formalize this simple idea with the help of Figure 1. It depicts the common pattern of a diversifying phylogenetic tree, but also can represent the pattern of shared feature diversity, with branch lengths representing the number of unique features that have evolved along that lineage. Pruning the tree illustrates how species and groups of species may differ in the number of unique features they have: if we prune distinctive species (such as the [Aardvark] in Figure 1) we will lose more of the tree (more unique features) than if we prune less distinctive species (e.g. [Cat]). Likewise, if we prune species [Dog, Cat], we will lose more of the tree than if we prune [Beaver, Mouse 2], because, on the tree, species [Mouse 1] preserves much of the feature diversity represented by [Beaver, Mouse 2]. We can say that each of [Beaver, Mouse 1, Mouse 2] are redundant with respect to the features represented by the rodent lineage that gave rise to them.

The mouse-aardvark comparison and Figure 1 allow us to consider a set of interesting observations about how feature diversity is distributed across biodiversity, and what the impacts of historically and prehistorically recent (i.e. Holocene) extinctions have been on this distribution. First, it is now well known that biodiversity is very unevenly distributed across the tree of life: the fact that at the ordinal level the mammals are ~50% rodents (Rodentia) and 0.02% aardvark

(Tubulidentata) is typical (reviewed in Purvis and Hector, 2000). The few living monotremes are another obvious case within mammals, being only distantly related to the remaining ~5500 known Holocene species. In phylogenetic terms, the tree of life is very imbalanced, because clades differ greatly in the net diversification they have experienced. Interestingly, the simplest null models of diversification also lead to imbalance, though usually not as extreme as those observed in nature (reviewed in Mooers et al., 2007).

The second observation, made most clearly by Nee and May (1997), is that, at least under simple null models of diversification, random loss of species has a mild impact on the total loss of the tree. This is easy to intuit: if loss is random, more species will be chosen from the most speciose parts of the tree, which are those parts that contain the most redundancy. From the point of view of conserving the products of evolution in the face of current extinctions, this observation is often cited with some relief (see, e.g. Avise, 2005). Importantly, Nee and May (1997) highlight that the actual amount of evolutionary history lost depends strongly on the shape of the tree: the more imbalanced the tree, and the shorter the branches near the root, the more that can be lost (Vazquez and Gittleman, 1998; Heard and Mooers, 2000). Interestingly, Rausch and Bar-Yom (2004) applied diversification models similar to Nee and May's to make a very different claim: the distribution of genetic redundancy (for them, within species) is highly skewed, such that some lineage (individuals) would be genetically much more distinct than others – i.e. *nonrandom* loss of these lineages would have a

large effect on total genetic diversity. This perspective of nonrandom loss is very important, and the question of the fail-safe of phylogenetic redundancy becomes largely empirical. Heard and Mooers (2000) documented that (i) though nonrandom loss alone may contribute little to loss overall, (ii) nonrandom loss concentrated within slowly-diversifying groups could lead to very large losses.

The Nee and May result spurred a series of empirical investigations into how much of the tree of life would be lost if current extinction risk projections were predictive: von Euler (2001) considered birds of the world, Purvis et al. (2000) considered both the world's birds and the world's carnivores and primates, Sechrest et al. (2002) looked at these latter two groups in the world's biodiversity hotspots, Johnson et al. (2002) studied marsupials in Australia, Russell et al. (1998) looked at historical and projected extinctions for all mammals and birds (using taxonomies), and Mooers and Atkins (2003) considered at-risk birds in Indonesia. All these studies agreed that projected losses are significantly greater than if extinctions were random, because projected losses are clumped and/or concentrated in species-poor groups. In addition, von Euler (2001) offered evidence that projected extinctions will make the future bird tree even less balanced than it is today. This means that feature diversity will be even less uniformly distributed among living species than at present, increasing the distinctiveness of some remaining lineages.

It might help to contextualize the main observation (nonrandom loss of feature diversity) by comparison with past extinctions, and this is the focus of this final chapter. For this we must move from time-based model trees to primarily morphology-based taxonomies, and this presents several interesting issues. Phylogenetic trees are often depicted with all the species an equal distance from their common root, so that we can think of branch lengths as equal to time. Elapsed time must be correlated with feature diversity (Crozier, 1997). However, we know of no good quantitative test of the strength of this correlation, partly because the very notion of feature diversity is vague. Williams and Gaston (1994) argue clearly that the correlation may be low, and indeed there is no theoretical reason to expect that morphological evolution should proceed at a constant rate (this is the reason why molecular clocks based on neutral genetic mutations are used instead of cumulative phenotypic character state changes to estimate phylogenetic divergence dates). So, for example, *Amborella trichopoda* seems to be the sister group to all other living flowering plants (Mathews and Donogue, 1999). As such it is a monotypic lineage on a very long branch of the tree of life. However, it is presented in textbooks as classically 'primitive', representing a suite of 'basal' characters rather than a suite of novel ones: time seems to have stood still for this particular lineage. *Sphenodon punctatus* (tuatara), is another case. Recent genetic studies (Hay et al. 2008) suggest that although morphologically unchanged since the Cretaceous, *S. punctatus* may in fact possess one of the fastest rates of molecular evolution for the mitochondrial DNA control region ever observed, making a strong case that although

morphologically ‘primitive’ it might be inappropriate to label *S. punctatus* as ‘primitive’ in a phylogenetic context. *S. punctatus* and *A. trichopoda* are only two examples of many such small-taxon number lineages, and considerable quantitative work still needs to be done to define and understand the relationships between feature diversity and phylogenetic diversity. Furthermore, it is important to bear in mind that there are two different hypotheses to explain the existence of small taxonomic groups today.

Some small taxa (e.g. obligate river dolphins, most of which represent monotypic families) have persisted for considerable periods but have not diversified further, presumably as a result of the diminished likelihood of diversification under certain ecological settings (see Vrba 1984). This long persistence at low species diversity provides no intuitive reason to suspect any increased vulnerability to extinction. Conversely, other small taxa were formerly very species-rich (e.g. sloths; see e.g. Chapter *ref at proof stage*, and Kurtén and Anderson 1980), but have experienced disproportionately high levels of extinction. If extinction risk is phylogenetically patterned (see, e.g. Purvis et al., 2000), then species from these taxa may be at increased risk of extinction in the future. However, these two different kinds of small taxa are generally not differentiated in analyses of extinction risk and further research is again required to quantify their relative contributions to present-day feature diversity.

Holocene extinctions.-

Here, we use taxonomies as surrogates for phylogenetic trees and in the case of mammals the recently published supertree for all mammals (Bininda-Emonds et al., 2007) to examine how Holocene extinctions were distributed among taxa.

We begin by examining taxonomies, a classification system based primarily on morphological differences. Taxonomies may offer a crude compound measure of feature diversity and time – insofar as they can often be interpreted as reflections of underlying phylogeny (especially following the incorporation of cladistic methodology into taxonomy from the mid twentieth century onwards). New taxa are probably more likely to be recognised when groups of individuals or species are phenotypically distinct (Scotland and Sanderson, 2003), and so a taxonomy must contain at least some information about how feature diversity is shared among its members. We recognize, however, that until we have a better concept of feature diversity, this argument is weak (e.g. *A. trichopoda* had already been assigned to its own order before the phylogenetic work was done and it is different because it is primitive, not different because it is derived.)

Our point of departure is the study by Russell et al. (1998) on the taxonomic patterns of recent (post-1600) and projected bird and mammal extinctions. We ask the same two questions they did: were Holocene extinctions nonrandom in that they were concentrated in particular subtaxa, and, if so, were these subtaxa

small? The first pattern would point to biological correlates of extinction risk, but would not lead to an appreciable loss of feature diversity (see, e.g. Figure 3 in Heard and Mooers, 2000), whereas the second pattern could lead to substantial losses of higher-order taxa (see, e.g. Figure 4 in Heard and Mooers, 2000). Using the recently published mammalian supertree (Bininda-Emonds et al. 2007), we examine these same two questions by looking at the change in imbalance from a reconstructed Holocene tree to the pruned-by-extinction current tree. Nonrandom loss that is not clustered within small taxa might have little effect to the overall balance of the tree (but see Heath et al., 2007), while nonrandom losses clustered within small taxa would increased imbalance, at least until a tipping point is surpassed where monotypic taxa are completely removed from the pruned phylogeny, rendering the tree more balanced.

We made use of the taxonomy and lists of mammals and birds known to have gone extinct in the past 11,000 years presented earlier in this volume (chapter *ref at proof stage*) cross-checked with the IUCN 2007 Redlist (IUCN, 2007), Mammal Species of the World, volume 3 (Wilson and Reeder, 2005), and the Systema Naturae 2000 (Brands, 2007) online taxonomic database. This database, which lists many extinct taxa, was also important in checking synonymies for extinct and modern species. Because the taxonomic postion of extinct species are sometimes not fully resolved, the genera and family datasets are not fully nested. Our mammal dataset lists as extinct 249/5577 species, 70/1276 genera (with 16/5577 extinct species not assigned to a genus), 9/159

families and 1/14 orders (the enigmatic aardvark-like Bibymalagasia of Madagascar). Our bird dataset lists as extinct 520/10324 species, 89/2166 genera (with 38/10324 species not assigned to a genus), 11/204 families and 2/24 orders (Aepyornithiformes elephant birds from Madagascar and the Dinornithiformes Moas from New Zealand). This list is obviously not complete: for instance, an estimated but undocumented 2,000 flightless rail species may have gone extinct between 3,500 and 1,000 years BP in the South Pacific (Steadman, 1995; see Chapter *ref at proof stage* for further discussion).

Extinct species were added into the mammal supertree manually using the program PhyloWidget (Jordan, 2008; http://www.phylowidget.org/). The taxonomic position of each extinct species from primary literature and the Systema Naturae 2000 database were used to work out the sister taxa and depth of each node added. Only extinct species whose taxonomic position was fully resolved were added to the supertree (i.e. the 233 taxa used in the genus level analysis).

A first reasonable question is whether ~5% of 5500 mammal (or of 10 000 bird species) is an anomalous number to lose over 11 000 years. The following are some very rough calculations based on simple null models of the average tempo of diversification that might help (for a guide, see, e.g. Baldwin and Sanderson, 1998, Ricklefs, 2003). We start with a constant-rate birth-death model of diversification,

$$n_t = n_0 e^{rt},$$

where $r = b-d$, b and d refer to instantaneous speciation and extinction rates per lineage, and n_0 = the number of species at time zero. If we set t_0 to 1.66 x 10^8 years ago (the deepest split in the placental mammal tree, Bininda-Emonds et al., 2007) when $n_0 = 2$, and if we set n_t= 5500 species of mammal (the total number in our dataset), then, by re-arrangement, we get r = [ln(5500)-ln(2)]/1.66 x 10^8 yr = 5 x 10^{-8}yr^{-1}. In order to try to achieve a high enough number of extinctions, we can set $d = 0.9b$ (a high turnover rate sometimes assumed in modeling diversification: see, e.g. Magallon and Sanderson, 2001), b would be approximately 5 x 10^{-7}yr^{-1} and d = 4.5 x10^{-7}yr^{-1}. If we now look at any time slice when many lineages are extant, then the overall rate of addition of new species is deterministically $n_0 b e^{rt}$, and so the total (deterministic) number of 'births' can be calculated as

$$\text{births} = n_0 \int_{t=0}^{t=t^*} b e^{rt} dt .$$

For n_0 = 5500 and t^*= 11000 yrs and the b and d estimates above, this is < 25, and the number of deaths would be less than that, i.e. << 0.5% of the standing crop. Increasing turnover further in order to achieve the observed ~240 Holocene extinctions, in line with the surely underestimated record number of mammal extinctions, requires that $d = 0.9875b$, but would imply a birth rate of 4 x 10^{-6} yr^{-1}. This in turn demands that the average species is only b^{-1} = 250 000

years old. Given that genetic evidence suggests that new species of vertebrate take on the order of 10 000 000 years to form (Avise and Walker, 1998), and an oft-quoted the average species age for mammals is 5 000 000 years (see, e.g. Purvis and Hector, 2000), this does not seem like a biologically reasonable turnover rate (see also Ricklefs, 2003). Calculations for birds lead to similar values. Even including preservation biases, estimates for average species ages taken directly from the fossil record are also on the order of a million years (Kemp, 1999). Taken together, the evidence suggests that the number of recorded mammal and bird extinctions in the Holocene is unusual and was not compensated for by the production of new species. This is not a controversial conclusion, as it is widely recognised that extinctions have occurred rapidly over ecological rather than evolutionary timescales during the late Quaternary. In addition, no Holocene species-level bird or mammal extinctions are considered to represent 'natural' (i.e. non-anthropogenic) events (see, e.g. Turvey, this volume). These prehistoric late Quaternary extinctions are interpreted as the beginning of a mass extinction event, comparable to those observed in the Phanerozoic fossil record, and which is ongoing today.

In order to investigate the taxonomic patterning of these extinctions, we followed the clear procedures outlined in Russell et al. (1998). We scored the size n_i of each taxon *i* (genus or family), and the proportion of species extinct and extant in each. Standard binomial theory allows us to produce numbers of species one would expect to go extinct in taxa of a given size and so the expected number of

taxa of each size that would be lost entirely. If extinction were random, then the observed proportion of extinct species *p* is our maximum likelihood estimate of the global probability of extinction. For the taxonomically assigned mammals, p = 0.042 and for taxonomically assigned birds, p = 0.047. If the number of taxa of each size *n* is S_n, then the expected number of taxa of S_n that are wholly extinct is just $S_n\, p^n$. This expectation will have a standard deviation of $[S_n p^n(1\text{-}p)^n]^{1/2}$. This pattern of random extinction is presented in the grey bars in Figure 2 and compared with the observed number of extinct taxa. Overall, if we take two standard deviations above the expected value as our guide, roughly twice as many higher taxa than expected under a random extinction scenario have been lost throughout the Holocene (e.g., 70 observed mammal genera gone versus a maximum of 24 expected, 9 observed vs. 1 to 2 expected extinct mammal families, 89 vs. 43 expected extinct bird genera, and 11 versus 1 to 2 expected extinct bird families).

Russell et al. (1998) speak of 'selectivity by size', where probability of extinction is some smooth function of taxon size. Using the standard R-package statistical library (www.r-project.org), and assuming binomial error, we used maximum likelihood to fit our data using the same generalized non-linear model as Russell et al.,

$$p_i = \frac{e^{(b_0 + b_1 \ln(n_i))}}{1 - e^{(b_0 + b_1 \ln(n_i))}},$$

where p_i is the proportion of species extinct in taxon *i* and n_i is its size, and b_o and b_1 are fitted parameters. Figure 3 plots the fitted lines of the binomial models of p_i on ln(n_i) and also the average proportion extinct for taxa of size ln(*n*). For both mammal and bird genera, and for bird families, there is a significant effect of taxon size on p_i, such that smaller taxa were more likely to lose species to extinction (see Figure 3 legend for values). Outliers in Figure 3 are individual taxon *sizes* that are ill-fit by the size-selectivity model.

We then used these fitted equations to produce a set of p_n, the probability of extinction for each taxon of size *n*. This can then be substituted into the simple binomial equations above to produce the expected number of taxa, and associated standard deviations, that would go extinct if there were only selectivity by size. These are depicted in the open bars in Figure 2. Taxa sizes that are not well-fit by this equation are those for which there has been some 'selectivity by taxon,' i.e. clumping or dispersion of extinction that is not predicted by taxon size. Risk to monotypic taxa is well-described by size selectivity. However, for both mammal and bird genera, taxa with 2-5 members are at higher risk even than expected based on their size, and a few entire larger-sized genera have also been wiped out, consistent with taxon selectivity not predicted by size. The same general patterns hold at the family level: while monotypic families are well-fit by the size-selectivity model, taxa of size 2, and the three extinct large-bodied families of Paleognathes (Aepyornithidae (elephant birds), Dinornithidae (giant

moas) and Emeidae (smaller moas)) that radiated on islands show selectivity not predicted by taxon size.

Overall, these patterns we report are similar those reported by Russell et al. (1998) for historical extinctions and extinctions projected by IUCN Red List data. What contributes to this clumping of extinctions within taxa? Holocene extinctions and the subset considered 'historical' (post-1600) by Russell et al. exhibit a strong taxon-size bias, where smaller taxa are more likely to be affected. Consistent with Russell et al.'s IUCN projections, birds show a weaker taxon-size effect than do mammals for genera. Russell et al.'s preferred explanation for this difference was that mammals in larger taxa were understudied, leading to a bias. However, this does not seem to hold here – if anything, we might have better data for mammal than for bird Holocene extinctions. Another possibility may be that the increased dispersal capabilities of birds has led to a difference in the taxonomic distribution of island-dwelling birds versus mammals, with bird taxa tending to contain both (more often extinct) island and (more often extant) mainland taxa. In birds, extent of annual dispersal and diversification rate are positively correlated (Phillimore et al., 2006). If high dispersing (and more species-rich) taxa contain species more likely to reach islands, this would weaken (or even reverse) a negative relationship between taxon size and extinction probability.

Superficially, patterns of modern extinctions are not overwhelmed by a contrasting pattern if we extend back over the Holocene. Table 1 and Figure 3 highlight that there was also a strong taxon-size independent component of selectivity: many taxa are ill-fit by the taxon-size selectivity curve. We identified individual taxa with too many extinct species as those that have lost at least Round($n_i p_n$+($2n_i p_n(1-p_n)$)$^{1/2}$), and these are listed in the Appendix.

These two types of taxonomic clumping of extinction can be further disentangled by considering a further quantity, the number of taxa of each size affected (i.e. taxa with at least one extinction; Russell et al., 1998). The observed numbers can be compared with expected numbers both under a global p (equal to $S_n(1-(1-p)^n)$ and the expected numbers under the size-selective model (equal to $S_n(1-(1-p^n)^n)$). These numbers are presented in Table 2, and summarized in Figure 4 alongside the total numbers of entire taxa lost and the total expected. The logic, as presented by Russell et al., is straightforward. Selectivity by taxon size (where species in smaller taxa are more likely to be extinct) results in more taxa lost, but can offer a form of compensation in that fewer taxa are affected. However, extreme clumping in the smallest taxa can have the opposite effect (e.g. if every extinction were in a monotypic genus, then the number of taxa affected would equal the total number of extinctions, much higher than any random expectation; Russell et al., 1998). If only large taxa were hit with extinctions, or at least if non-size selectivity is concentrated in relatively larger taxa, then fewer taxa might be expected to be lost, with fewer taxa affected. This

might be considered the ideal if we are interested in the preservation of evolutionary feature diversity. All four groups show a consistent pattern: size selectivity means more entire taxa are lost than expected, but selectivity in some larger-sized taxa means that there are fewer taxa affected than one would expect from the size-effect model alone. In other words, many of the taxa that are outliers in Figure 3 by virtue of having no recorded extinctions are the result of extinctions being clumped elsewhere. This also brings up a critical issue of scale: whether clumped extinction leads to the overall loss of feature diversity depends on how those features are distributed among taxa through the tree. Our implicit interpretation here is that more diversity is distributed among families than among genera within families. Null models of diversification and feature evolution may offer a guide here, but more empirical work is also needed.

We augment these analyses with a preliminary look of the change in the shape of the mammal supertree (Bininda-Emonds et al. 2007) following Holocene extinctions. We made use of a measure of tree shape, I_w, which allows the inclusion of polytomies (Fusco and Cronk, 1995, modified by Purvis et al., 2002). This measure has an expectation of 0.5 under the simplest null model of random diversification, and approaches 1.0 as trees become more imbalanced. We compared the change in shape from the loss of 233 extinct species that we could place on the mammal supertree with the expectation if such losses were taxonomically random.

Not surprisingly, the Holocene mammalian phylogeny appears imbalanced (I_w > 0.5 = 0.633). When extinct taxa are pruned from the tree imbalance increases (I_w = 0.645), and this increase is significant ($p < 0.05$) relative to the null expectation of random loss ($E(I_w) = 0.628$, s.d. = 0.008, n = 1000 bootstraps). In agreement with the taxonomic work, the increase in imbalance is most likely due to non-random losses from species poor clades over the course of the Holocene.

The explanation for the non-random loss of species from small taxa during the Holocene is most certainly the island effect. First, small-range species have experienced the great majority of post-glacial anthropogenic extinctions (for passerine birds, see Manne et al., 1999), and island species tend to have small ranges (usually by virtue of geographic isolation of insular populations). Indeed, 207/249 of the extinct mammal species and fully 495/520 extinct bird species were either endemic to islands or geographically restricted to islands or island systems by the start of the Holocene. The three extinct orders were also each endemic to one of two island systems (Madagascar and New Zealand). Island species tend to be placed in their own taxonomic groups, perhaps due to divergent selection imposed by island habitats, or by predetermined biases in taxonomic classification. It is important also to recognise that island systems can act both as refugia for ancient, typically species-poor lineages (e.g. tuatara) and also as centres of evolutionary radiations of more recent colonists (e.g. drepanidine honeycreepers and *Drosophila* in the Hawai'ian archipelago). However, both sets of species appear to be similarly vulnerable to anthropogenic

impacts as they have typically evolved in the absence of many native predators (notably mammalian predators). It remains unclear whether there is any relationship between relative age and size of island taxa and their vulnerability to extinction. Following on from the work reported here, it would be interesting to compare patterns of taxon size selectivity and extinction in the earlier late-Pleistocene extinctions, which had a continental rather than insular focus but which also clearly impacted many mammals that survive today only as small taxa; however, this is beyond the scope of the present volume.

Holocene effects on present-day distributions.-

Humans have had a significant impact on global biodiversity during the Holocene. The extinctions recorded here and projected extinctions based on IUCN Red List data (Russell et al., 1998) have had, and likely will lead to, a greater than random loss of feature diversity, at least for birds and mammals (the best-studied taxonomic groups for which we currently possess the most meaningful data). In addition, past taxon selectivity could produce present day small, at-risk taxa. For example, the two living members of the family Elephantidae (*Elephas maximus* and *Loxodonta africana*) that express the unique feature diversity of the entire classical order Proboscidea are the remnants of a clade with at least 10 members that still survived after the Last Glacial Maximum in the terminal Pleistocene. These two remaining species are

also at fairly high risk of extinction: *L. africana* is classified as Vulnerable and *E. maximus* is classified as Endangered by IUCN (2007).

Is it possible that past anthropogenic extinction has created extreme skew in present-day feature diversity? Eleven of the top 100 most evolutionarily distinctive mammals have had close (confamilial) relatives lost in the Holocene, increasing their taxonomic and phenotypic isolation. Besides the two elephant species, the list includes the two remaining hippopotamus species *Hexaprotodon liberiensis* and *Hippopotamus amphibius*, the aye aye *Daubentonia madagascarensis*, the greater bilby *Macrotis lagotis*, the two solenodons *Solenodon cubanus* and *S*. *paradoxus*, the dugong *Dugong dugon*, the steppe Pika *Ochotona pusilla* and the New Zealand lesser short-tailed bat *Mystacina tuberculata*. Disconcertingly, all 11 of these species are also listed as globally threatened by the IUCN. A combination of evolutionary distinctiveness and global threat is encapsulated in the Edge of Existence list administered by the Zoological Society of London (ZSL). Nine of these newly-distinctive species are on the ZSL's top-100 "Edge of Existence" mammal list (and the missing two, *H. amphibius* and *M. lagotis*, ranked 137th and 130th respectively). It may be that many of the species now listed by this innovative program may be there as a result of human precipitated extinctions of close relatives within the last 11 000 years. However, connexions among past losses, current threat and current distinctiveness would need to be investigated more formally. So, while many of the top 100 EDGE species are insular, the Spearman rank correlation between

threat status and evolutionary distinctiveness, as measured by Isaac et al. (2007, their supplementary material) while significantly positive, has very low explanatory power ($\rho = 0.05$, $p = 0.001$, $n = 4507$).

In conclusion, not only have Holocene mammal and bird extinctions occurred at a significantly elevated rate, but taxa containing disproportionately few species are both disproportionately threatened with extinction today (Russell et al., 1998) and have also experienced elevated rates of species loss over the past 11 000 years (our results) as well as farther back in time (McKinney, 1997). We end this short chapter by noting that it is not immediately obvious how to evaluate the importance of the non- random loss of feature diversity through the Holocene that we document here. If the same number of extinctions had taken place but they had been random with respect to the tree of life, would the world be better off? Implicit in the research agenda that looks at the loss of feature diversity through extinction is the idea that species are of different value: to quote George Orwell, "all animals are equal but some animals are more equal than others". But it need not be true from first principles that more feature diversity as we are measuring it is much better in any ecologically or evolutionarily meaningful way. For example, the species-richness – ecosystem function debate (Cardinale et al., 2006; Worm et al., 2006) is ongoing. We need more work on the relationship between feature diversity and phylogenetic diversity. We also need more work on the use and nonuse values of each.

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Figure legends

Figure 1. Schematic diagram demonstrating clumping of extinction on a phylogenetic tree. Branch lengths represent the number of unique features. The loss of Aardvarks would lead to the greatest loss of feature diversity. If both Dog and Cat go extinct, their shared feature diversity (dashed branch) would also be lost. Extinction of Beaver and Mouse 2 would lead to less loss, because Mouse 1 would represent the shared features of the set (dotted branches).

Figure 2. The numbers of extinct Holocene taxa of a given size. Dark bars indicate the actual number, grey bars are the expectations if extinction were random, and open bars the expectation under size selectivity (from models presented in Figure 3). Error bars depict two standard deviations under a binomial distribution. Panels are standard for all subsequent figures: top left panel depicts mammal genera; top right panel depicts bird genera; bottom left panel depicts mammal families; and the bottom right panel depicts bird families.

Figure 3. Non-linear fits of the proportion of species extinct in a taxon as a function of its size: $p_i=\exp(B_0+B_1*\ln(n_i))/ (1- \exp(B_0+B_1*\ln(n_i))$. The datapoints are the average prop(extinct) for taxa of that size. Panels are arranged as in Figure 2. For mammal genera (top left), $B0 = -2.30$ ($P < 0.0001$) and $B1 = -0.38$ ($P = 0.005$). Corresponding values for the other datasets are as follows: bird genera (top right),$B0 = -2.61$ ($P < 0.0001$), $B1 = -0.20$ ($P = 0.05$); mammal families (bottom left), $B0 =-1.60$ ($P < 0.0001$), $B1=-0.30$ ($P = 0.11$); bird families (bottom right), $B0 = -1.38$ ($P = 0.0003$)$B1=-0.41$ ($P = 0.01$).

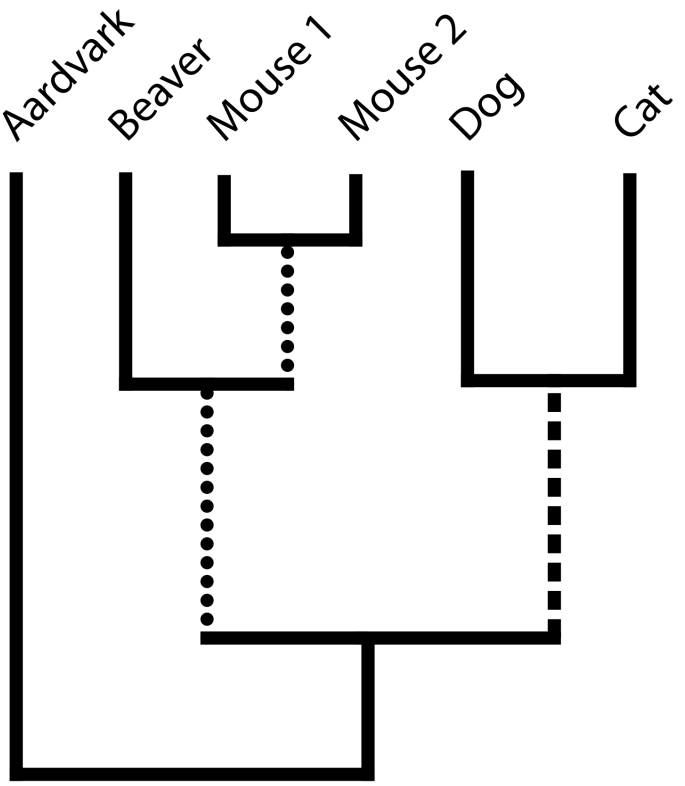
Figure 4. In closed bars are the total number of taxa expected to have been lost during the Holocene under taxonomically random extinction [sum across sizes n of $n*p^n$]; the total number under size-selective extinction [sum across sizes n of $n*p_n$], and the observed total number. Open bars depict the total number of taxa with at least one extinction during the Holocene under random extinction [sum across sizes n of $n(1-(1-p)^n$], the total under the size selectivity model [sum across sizes n of $n(1-(1-p_n)^n)$]; and the observed total. Panels are arranged as in Figures 2 and 3, with genera above and mammals to the left.

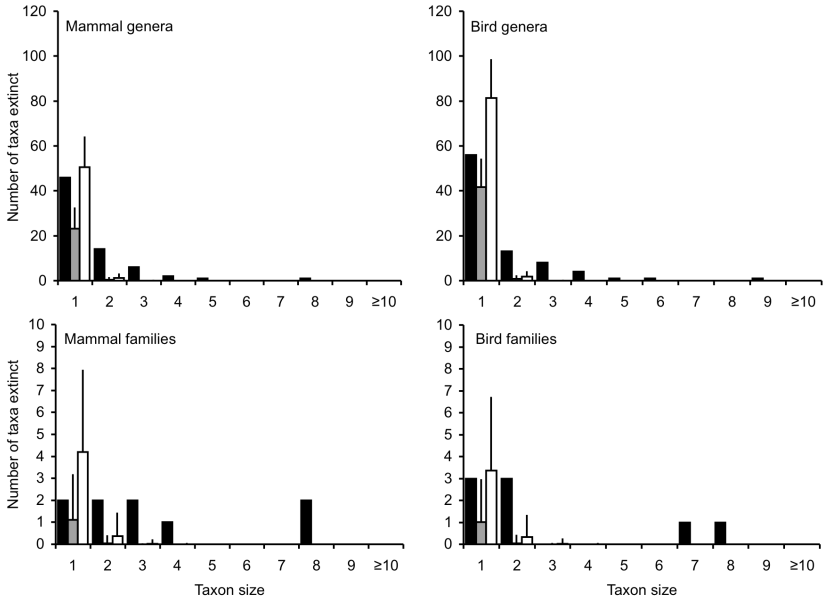
Table 1. Number of recorded Holocene extinct 'groups' per group size for mammal and bird genera and families

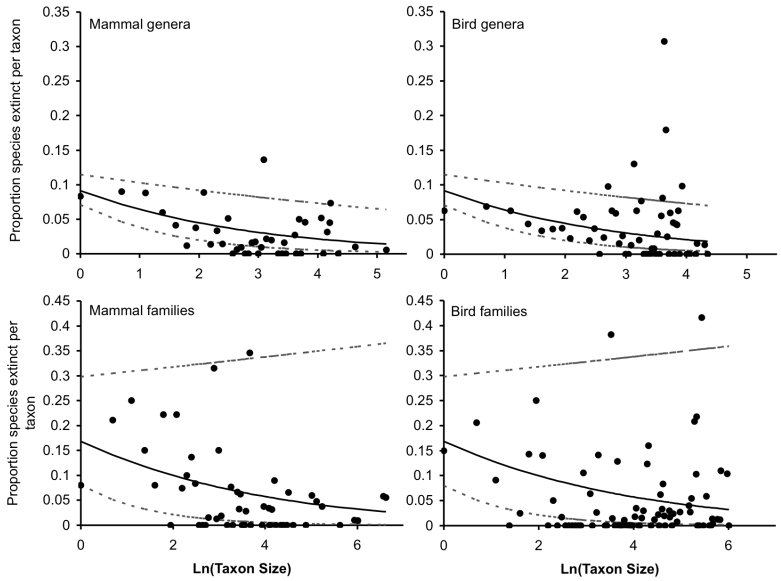
group		group size	#groups	p_n	actual	predicted	2 S.D.
mammals	genus	1	555	0.091	46	50.628	13.566
		2	234	0.072	14	1.198	2.033
		3	110	0.062	6	0.026	0.294
		4	92	0.056	2	0.001	0.053
		5	53	0.052	1	0.000	0.008
		6	28	0.048	0	0.000	0.001
		7	19	0.046	0	0.000	0.000
		8	31	0.043	1	0.000	0.000
		9	25	0.042	0	0.000	0.000
		10+	129	0.028	0	0.000	0.000
	family	1	25	0.168	2	4.207	3.741
		2	19	0.141	2	0.379	1.057
		3	8	0.127	2	0.016	0.209
		4	10	0.118	1	0.002	0.068
		5	5	0.111	0	0.000	0.014
		6	3	0.106	0	0.000	0.003
		7	6	0.101	0	0.000	0.001
		8	9	0.098	2	0.000	0.000
		9	3	0.095	0	0.000	0.000
		10+	71	0.061	0	0.000	0.000
birds	genus	1	896	0.068	60	61.106	15.092
		2	347	0.060	12	1.247	2.099
		3	211	0.056	7	0.036	0.349
		4	151	0.053	5	0.001	0.061
		5	95	0.050	2	0.000	0.010
		6	63	0.049	1	0.000	0.002
		7	57	0.047	1	0.000	0.000
		8	35	0.046	0	0.000	0.000
		9	46	0.045	1	0.000	0.000
		10+	265	0.036	0	0.000	0.000
	family	1	21	0.200	4	4.206	3.668
		2	18	0.158	4	0.451	1.130
		3	13	0.137	0	0.034	0.294
		4	7	0.124	0	0.002	0.062
		5	8	0.114	0	0.000	0.018
		6	6	0.107	0	0.000	0.004
		7	4	0.101	1	0.000	0.001
		8	8	0.096	1	0.000	0.000
		9	2	0.092	0	0.000	0.000
		10+	117	0.044	1	0.000	0.000

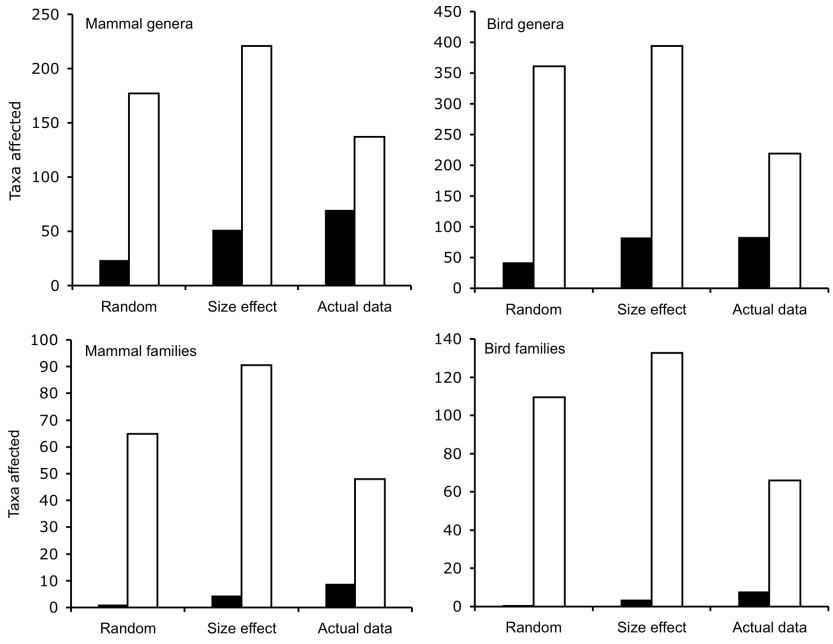
Table 2. Number of groups of mammal and bird genera and families with ≥1 recorded extinct Holocene species.

group		group size	# groups	actual	p_n	predicted	2 S.D.
mammals	genus	1	555	46	0.091	50.628	13.566
		2	234	28	0.138	32.290	3.639
		3	110	15	0.175	19.201	1.147
		4	92	10	0.205	18.900	0.511
		5	53	6	0.233	12.323	0.196
		6	28	1	0.257	7.189	0.074
		7	19	1	0.279	5.298	0.032
		8	31	4	0.299	9.274	0.022
		9	25	2	0.318	7.950	0.010
		10+	129	24	0.560	1.809	0.000
	family	1	25	2	0.168	4.207	3.741
		2	19	6	0.262	4.985	1.687
		3	8	2	0.335	2.678	0.594
		4	10	2	0.394	3.942	0.361
		5	5	1	0.445	2.224	0.136
		6	3	1	0.488	1.465	0.054
		7	6	0	0.527	3.162	0.038
		8	9	2	0.561	5.051	0.022
		9	3	2	0.592	1.775	0.006
		10+	71	30	0.895	1.358	0.000
birds	genus	1	896	60	0.068	61.106	15.092
		2	347	34	0.116	40.355	3.829
		3	211	19	0.158	33.252	1.405
		4	151	11	0.194	29.369	0.603
		5	95	9	0.228	21.671	0.254
		6	63	5	0.259	16.325	0.112
		7	57	7	0.288	16.413	0.059
		8	35	7	0.315	11.021	0.026
		9	46	10	0.340	15.650	0.016
		10+	265	61	0.670	2.950	0.000
	family	1	21	4	0.200	4.206	3.668
		2	18	5	0.291	5.246	1.752
		3	13	3	0.358	4.649	0.794
		4	7	0	0.410	2.872	0.310
		5	8	1	0.454	3.632	0.173
		6	6	1	0.492	2.949	0.076
		7	4	1	0.524	2.097	0.031
		8	8	2	0.553	4.425	0.021
		9	2	0	0.579	1.158	0.005
		10+	117	51	0.907	1.348	0.000


Aardvark
Beaver
Mouse 1
Mouse 2
Dog
Cat


Mammal genera
Bird genera
Mammal families
Bird families
Number of taxa extinct
Taxon size
1
2
3
4
5
6
7
8
9
≥10


Mammal genera
Bird genera
Mammal families
Bird families
Proportion species extinct per taxon
Proportion species extinct per taxon
Ln(Taxon Size)
Ln(Taxon Size)


Mammal genera
Bird genera
Mammal families
Bird families
Taxa affected
Taxa affected
Random
Size effect
Actual data
250
200
150
100
50
0
450
400
350
300
250
200
150
100
50
0
100
90
80
70
60
50
40
30
20
10
0
140
120
100
80
60
40
20
0

Appendix 1: List of taxa having undergone more extinctions than expected. Families listed in bold have suffered higher than expected numbers of extinct species. Each taxa is listed with the number of extinct species / total number of species in that taxa. § – extinct Order; ‡ – extinct Family; † – extinct Genus.

A) MAMMALS	**genus**	**family**
Artiodactyla		
Bovidae		
Bubalus	2/6	
Hippopotamidae		4/6
Hippopotamus	3/4	
§Bibymalagasia		
‡Plesiorycteropodidae		2/2
†Plesiorycteropus	2/2	
Carnivora		
Canidae		
†Dusicyon	2/2	
Chiroptera		
Phyllostomidae		
Desmodus	3/4	
Phyllostomidae		
Phyllops	2/3	
Pteropodidae		
Pteropus	6/67	
Dasyuromorphia		
‡Thylacinidae		2/2
†Thylacinus	2/2	
Diprotodontia		
Macropodidae		
Lagorchestes	2/4	
Pilosa		
Megalonychidae		16/18
†Acratocnus	4/4	
†Megalocnus	2/2	
†Neocnus	5/5	
†Parocnus	2/2	
Primates		
‡Archaeolemuridae		3/3
†Archaeolemur	2/2	
Lemuridae		
†Pachylemur	2/2	
‡Megaladapidae		3/3
†Megaladapis	3/3	
‡Palaeopropithecidae		8/8
Rodentia		
Capromyidae		27/39
Capromys	6/8	
Geocapromys	5/7	
†Isolobodon	2/2	
Mesocapromys	8/12	
Plagiodontia	2/3	
Cricetidae		41/710
†Megalomys	4/4	
†Megaoryzomys	2/2	
Neotoma	3/22	
Nesoryzomys	6/8	
Echimyidae		
†Boromys	2/2	
†Brotomys	2/2	
Gliridae		
Eliomys	2/5	
‡Heptaxodontidae		4/4
Muridae		42/754
†Canariomys	2/2	
†Coryphomys	2/2	
†gen. 1	3/3	
†gen. 2	3/3	
†gen. 4	2/2	
Notomys	5/10	
Rattus	5/68	
Soricomorpha		
‡Nesophontidae		8/8
†Nesophontes	8/8	
Solenodontidae		
Solenodon	2/4	
Soricidae		
†Asoriculus	3/3	

†Mesopropithecus	3/3	
†Palaeopropithecus	3/3	

B) BIRDS	**genus**	**family**
§Aepyornithiformes		
‡Aepyornithidae		7/7
†Aepyornis	4/4	
†Mullerornis	3/3	
Anseriformes		
Anatidae		41/197
Alopochen	3/4	
Anas	6/48	
Branta	4/10	
†Cnemiornis	2/2	
Cygnus	3/9	
†Thambetochen	2/2	
Ciconiiformes		
Accipitridae		15/255
Aquila	3/15	
†Titanohierax	2/2	
Ardeidae		12/75
Nycticorax	8/10	
Falconidae		9/73
Caracara	3/5	
Milvago	2/4	
Procellariidae		17/96
Pterodroma	7/38	
Puffinus	7/26	
Scolopacidae		14/102
Coenocorypha	5/7	
†Prosobonia	6/6	
Threskiornithidae		
†Apteribis	3/3	
Columbiformes		
Columbidae		38/345
Alectroenas	2/5	
Ducula	5/42	
Gallicolumba	6/24	
Craciformes		
Megapodiidae		13/34
Megapodius	11/23	
Cuculiformes		
Cuculidae		
Coua	3/12	
§Dinornithiformes		
Galliformes		
Phasianidae		
Coturnix	3/11	
Gruiformes		
‡Aptornithidae		2/2
†Aptornis	2/2	
Rallidae		96/231
†Aphanapteryx	2/2	
Atlantisia	2/3	
Fulica	4/15	
Gallirallus	28/38	
†Nesotrochis	3/3	
†Pareudiastes	2/2	
Porphyrio	10/15	
Porzana	26/39	
Rallus	3/12	
Gruidae		
Grus	4/17	
Passeriformes		
Acanthisittidae		4/6
Corvidae		
Corvus	8/51	
Fringillidae		45/207
†Aidemedia	3/3	
†Akialoa	9/9	
†Chloridops	3/3	
†Ciridops	4/4	
†Drepanis	2/2	
Loxoides	2/3	
†Rhodacanthis	4/4	
Telespiza	4/6	
†Vangulifer	2/2	
†Xestospiza	2/2	
Meliphagidae		
†Chaetoptila	3/3	
†Moho	5/5	
Monarchidae		
Pomarea	4/9	
Turdidae		
Myadestes	3/14	
‡Turnagridae		2/2
†Turnagra	2/2	
Sturnidae		
Aplonis	5/26	
Psittacidae		41/394

‡Dinornithidae		2/2
†Dinornis	2/2	
‡Emeidae		8/8
†Eurapteryx	2/2	
†Pachyornis	3/3	

B) BIRDS (continued)	**genus**	**family**
Strigiformes		
Caprimulgidae		
Siphonorhis	2/3	
Strigidae		21/204
Athene	4/7	
†Gallistrix	4/4	
†Mascarenotus	3/3	
†Ornimegalonyx	2/2	
Tytonidae		11/26
Tyto	10/23	
Struthioniformes		
Casuariidae		
Dromaius	2/3	

Amazona	6/37
Ara	8/16
Eclectus	2/3
Nestor	2/4
Psittacula	4/17
Vini	2/7

Appendix 2: Notes on the taxonomic database used. The dataset is that of Turvey (Chapter ##) for mammals and Tyrberg (chapter ##) for birds. Revisions and exclusions are listed below for mammals and birds. Revisions to the taxonomies were only made when extant taxa listed in the IUCN 2007 redlist where affected by the taxonomic discrepancies between datasets.

1) MAMMALS

Taxonomic Revisions	
Rodentia	
Cricetidae	
Microtus	Genus moved to Cricetidae from Muridae, to be consistent with extant species listed in IUCN 2007.

Taxa excluded from generic analysis
Rodentia
Cricetidae
Oryzomyini gen. et sp. indet
sp. 1 - 14
Muridae
Melomys/Pogonomeiomys sp. nov. A
Melomys/Pogonomeiomys sp. nov. B

Taxa excluded from all analysis
Family Indet.
Tainotherium valei
Rodentia?
gen. et sp. nov.
Chiroptera
Family indet.
Boryptera alba

2) BIRDS

Taxonomic Revisions	
Columbiformes	
Columbidae	
Pezophaps solitaria	IUCN 2007 lists these taxa as Raphidae. We use Tyrberg's taxonomy, as no extant taxa are affected by the taxonomic changes.
Raphus cucullatus	

Gruiformes	
Rallidae	
Atlantisia elpenor	Species transferred to the genus Atlantisia from Mundia to be consistent with extant taxa and listings in IUCN 2007.
Pareudiastes pacifica	Species name changed to pacifica from pacificus to be consistent with IUCN 2007 listing.
Passeriformes	
Acanthisittidae	
Dendroscansor decurvirostris *Pachyplicas yaldwyni* *Traversia lyalli* *Xenicus longipes*	Genera moved to Acanthisittidae from Xenicidae to be consistent with the IUCN 2007. Acanthisittidae contains 2 extant species for which the relationship to Xenicidae was unclear, prompting the use of Acanthisittidae.
Fringillidae	
Psittirostra kona	IUCN 2007 lists this species as Chloridops kona. We use the taxonomy listed in Tyrberg's chapter. Three other extinct Chloridops species not listed in IUCN are included and considered Chloridops, consistent with Tyrberg's dataset.
Loxops sagittirostris	IUCN 2007 lists this species as Hemignathus sagittirostris. We use the taxonomy of Tyrberg (chapter).
Parulidae	
Leucopeza semperi	Genera moved to Parulidae from Emberizidae to be consistent with listing in IUCN 2007 listings.
Vermivora bachmani	Genera moved to Parulidae from Emberizidae to be consistent with extant taxa listed in IUCN 2007 listings.
Timaliidae	
Timaliinae undescribed gen. et sp.	Corrected spelling from Tamaliinae
Strutioniformes	
Casuariidae	
Dromaius ater *Dromaius baudinianus*	IUCN 2007 lists this genus as part of the Dromaiidae family. We use the taxonomy listed in Tyrberg's dataset. The extant Emu was revised to be consistent.

Taxa excluded from generic analysis

Anseriformes
- Anatidae
  - *aff. Anas undescribed species*
  - *aff. Tadorna undescribed sp.*
  - *Anatidae undescribed sp.*
  - *Anatidae "supernumerary Oahu goose"*
  - *cf. Dendrocgna undescribed sp.*

Charadriiformes
- Scolopacidae
  - *Coenocorypha? undescribed sp.*

Columbiformes
- Columbidae
  - *"Raperia"*
  - *godmanae*
  - *cf. Alectroenas undescribed sp.*
  - *Gallicolumba?*
  - *norfolciensis*
  - *undescribed gen. et sp.*
  - *undescribed gen. et sp.*
  - *A*
  - *undescribed gen. et sp.*
  - *B*
  - *undescribed gen. et sp.*
  - *C*

Falconiformes
- Falconidae
  - *Falconidae undescribed sml sp.*

Galliformes
- Megapodiidae
  - *Megapodiidae undescribed sp.*

Gruiformes
- Rallidae
  - *"Fulica"*
  - *podagrica*
  - *cf. Dryolimnas undescribed sp.*
  - *cf. Gallinula*
  - *sp.*
  - *cf. Porzana undescribed*
  - *sp.*
  - *Rallidae undescribed sp.*
  - *Rallidae undescribed sp.*
  - *A*
  - *Rallidae undescribed sp.*
  - *B*
  - *Rallidae undescribed sp.*
  - *C*
  - *Rallidae undescribed sp.*
  - *D*

Passeriformes
- Campephagidae
  - *cf. Lalage sp.*
- Meliphagidae
  - *cf. Chaetoptila undescribed sp.*
- Passeridae
  - *Foudia? undescribed sp.*
- Sylviidae
  - *cf. Cettia sp.*

- Timaliidae
  - *Timaliinae undescribed gen. et sp.*
- Turdidae
  - *Turdidae undescribed sp.*
- Zosteropidae
  - *Zosteropidae undescribed sp. 1*
  - *Zosteropidae undescribed sp. 2*

Psittaciformes
- Psittacidae
  - *"Necropsittacus" borbonicus*
  - *cf. Psittacidae undescribed sp. 1*
  - *cf. Psittacidae undescribed sp. 2*
  - *Psittacidae undescribed sp.*

Strigiformes
- Tytonidae
  - *Tyto? letocarti*

Taxa excluded from all analysis
- Aves incertae sedis
  - *"Aquila" simurgh*
- Passeriformes incertae sedis
  - *"Turdus" ulientensis*
  - *aff. Carduelis undescribed species*
  - *undescribed slender-billed species*

Appendix 3: taxa with unexpected numbers of extinct species

Mammal genera

ORDER	FAMILY	GENUS	Ng		# extinct sp / Gen
Artiodactyla	Hippopotamidae	Hippopotamus		4	3
Bibymalagasia	Plesiorycteropidae	Plesiorycteropus		2	2
Carnivora	Canidae	Dusicyon		2	2
Chiroptera	Mormoopidae	Pteronotus		8	2
Chiroptera	Phyllostomidae	Desmodus		4	3
Chiroptera	Phyllostomidae	Phyllops		3	2
Dasyuromorphia	Thylacinidae	Thylacinus		2	2
Diprotodontia	Macropodidae	Lagorchestes		4	2
Pilosa	Megalonychidae	Acratocnus		4	4
Pilosa	Megalonychidae	Megalocnus		2	2
Pilosa	Megalonychidae	Neocnus		5	5
Pilosa	Megalonychidae	Parocnus		2	2
Primates	Archaeolemuridae	Archaeolemur		2	2
Primates	Lemuridae	Pachylemur		2	2
Primates	Megaladapidae	Megaladapis		3	3
Primates	Palaeopropithecidae	Mesopropithecus		3	3
Primates	Palaeopropithecidae	Palaeopropithecus		3	3
Rodentia	Capromyidae	Capromys		8	6
Rodentia	Capromyidae	Geocapromys		7	5
Rodentia	Capromyidae	Isolobodon		2	2
Rodentia	Capromyidae	Mesocapromys		12	7
Rodentia	Capromyidae	Plagiodontia		3	2
Rodentia	Cricetidae	Megalomys		4	4
Rodentia	Cricetidae	Megaoryzomys		2	2
Rodentia	Cricetidae	Neotoma		22	3
Rodentia	Cricetidae	Nesoryzomys		8	6
Rodentia	Echimyidae	Boromys		2	2
Rodentia	Echimyidae	Brotomys		2	2
Rodentia	Gliridae	Eliomys		5	2
Rodentia	Muridae	Canariomys		2	2
Rodentia	Muridae	Coryphomys		2	2
Rodentia	Muridae	gen. nov. 1		2	2
Rodentia	Muridae	gen. nov. 2		2	2
Rodentia	Muridae	gen. nov. 4		2	2
Rodentia	Muridae	Notomys		10	5
Rodentia	Muridae	Rattus		70	7
Rodentia	Muridae	Solomys		5	2
Soricomorpha	Nesophontidae	Nesophontes		8	8
Soricomorpha	Solenodontidae	Solenodon		4	2
Soricomorpha	Soricidae	Asoriculus		3	3

Mammal families

ORDER	FAMILY	Nf	# extinct sp / Fam
Artiodactyla	Hippopotamidae	6	4
Bibymalagasia	**Plesiorycteropidae**	2	2
Dasyuromorphia	**Thylacinidae**	2	2
Pilosa	Megalonychidae	18	16
Primates	**Archaeolemuridae**	3	3
Primates	**Megaladapidae**	3	3
Primates	**Palaeopropithecidae**	8	8
Rodentia	Capromyidae	39	26
Rodentia	Cricetidae	712	43
Rodentia	**Heptaxodontidae**	4	4
Rodentia	Muridae	752	46
Soricomorpha	**Nesophontidae**	8	8

bird genera

FAMILY	GENUS	Ng	# extinct sp / Gen
Accipitridae	Aquila	15	3
Accipitridae	Titanohierax	2	2
Aepyornithidae	Aepyornis	4	4
Aepyornithidae	Mullerornis	3	3
Anatidae	Alopochen	4	3
Anatidae	Anas	49	7
Anatidae	Branta	10	4
Anatidae	Cnemiornis	2	2
Anatidae	Thambetochen	2	2
Aptornithidae	Aptornis	2	2
Ardeidae	Nycticorax	11	9
Caprimulgidae	Siphonorhis	3	2
COLUMBIDAE	Alectroenas	5	2
COLUMBIDAE	Gallicolumba	24	6
CORVIDAE	Corvus	51	8
CUCULIDAE	Coua	12	3
Dinornithidae	Dinornis	2	2
DROMAIIDAE	Dromaius	3	2
Emeidae	Pachyornis	3	3
Falconidae	Caracara	5	3
FALCONIDAE	Milvago	4	2
Fringillidae	Aidemedia	3	3
Fringillidae	Akialoa	9	9
Fringillidae	Chloridops	4	4
Fringillidae	Ciridops	4	4

FRINGILLIDAE	Drepanis	2	2
Fringillidae	Loxioides	3	2
FRINGILLIDAE	Rhodacanthis	4	4
Fringillidae	Telespiza	6	4
Fringillidae	Vangulifer	2	2
Fringillidae	Xestospiza	2	2
GRUIDAE	Grus	18	5
Megapodiidae	Megapodius	23	11
Meliphagidae	Chaetoptila	3	3
Meliphagidae	Moho	5	5
MONARCHIDAE	Pomarea	9	4
PHASIANIDAE	Coturnix	11	3
Procellaridae	Pterodroma	5	5
Procellaridae	Puffinus	7	7
PSITTACIDAE	Amazona	37	6
PSITTACIDAE	Eclectus	3	2
Psittacidae	Nestor	4	2
PSITTACIDAE	Psittacula	17	4
Psittacidae	Vini	7	2
RALLIDAE	Aphanapteryx	2	2
RALLIDAE	Atlantisia	3	2
Rallidae	Fulica	15	4
RALLIDAE	Gallirallus	37	27
Rallidae	Nesotrochis	3	3
Rallidae	Pareudiastes	2	2
Rallidae	Porphyrio	15	10
Rallidae	Porzana	39	26
Scolopacidae	Coenocorypha	7	5
Scolopacidae	Prosobonia	6	6
Strigidae	Athene	7	4
Strigidae	Grallistrix	4	4
STRIGIDAE	Mascarenotus	3	3
Strigidae	Ornimegalonyx	2	2
STURNIDAE	Aplonis	26	5
Threskiornithidae	Apteribis	3	3
TURNAGRIDAE	Turnagra	2	2
Tytonidae	Tyto	23	10

bird families

FAMILY	Nf	# extinct sp / Fam
ACANTHISITTIDAE	6	4
ACCIPITRIDAE	255	15
Aepyornithidae	7	7
Anatidae	197	41
Aptornithidae	2	2
ARDEIDAE	76	13
COLUMBIDAE	342	35

Dinornithidae	2	2
Emeidae	8	8
FALCONIDAE	73	9
Fringillidae	209	46
MEGAPODIIDAE	34	13
MELIPHAGIDAE	185	10
Procellaridae	95	16
Psittacidae	394	41
Rallidae	230	95
RAPHIDAE	2	2
SCOLOPACIDAE	102	14
STRIGIDAE	205	22
TURNAGRIDAE	2	2
Tytonidae	26	11

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