Investigating evolutionary processes using ancient and historical DNA of rodent species

Thesis submitted for the degree of Doctor of Philosophy (PhD)

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Declaration

I, Selina Brace, declare that this thesis and the work presented in it is entirely my own. Where I have consulted the work of others, it is always clearly stated.

Selina Brace   Ian Barnes

"Why should we look to the past?

……Because there is nowhere else to look."

James Burke

Abstract

The Late Quaternary has been a period of significant change for terrestrial mammals, including episodes of extinction, population sub-division and colonisation. Studying this period provides a means to improve understanding of evolutionary mechanisms, and to determine processes that have led to current distributions. For large mammals, recent work has demonstrated the utility of ancient DNA in understanding demographic change and phylogenetic relationships, largely through well-preserved specimens from permafrost and deep cave deposits. In contrast, much less ancient DNA work has been conducted on small mammals. This project focuses on the development of ancient mitochondrial DNA datasets to explore the utility of rodent ancient DNA analysis.

Two studies in Europe investigate population change over millennial timescales. Arctic collared lemming (*Dicrostonyx torquatus*) specimens are chronologically sampled from a single cave locality, Trou Al'Wesse (Belgian Ardennes). Two end Pleistocene population extinction-recolonisation events are identified and correspond temporally with - localised disappearance of the woolly mammoth (*Mammuthus primigenius*). A second study examines postglacial histories of European water voles (*Arvicola*), revealing two temporally distinct colonisation events in the UK.

Two further studies of Caribbean rodent species assess DNA recovery from more recent, yet highly degraded, material. The Hispaniolan hutia (*Plagiodontia aedium*), one of the few remaining endemic Caribbean land mammals, is found to represent three distinct lineages, biogeographically consistent with the island's Cenozoic origins and geographic heterogeneity. The extinct Antillean rice rats (*Oryzomyini*) were once endemic throughout the Lesser Antilles. Phylogenetic analysis of zooarchaeological rice rat material recovered from across the Lesser Antillean reveals a new genus of rice rat and at least two separate colonisation events, via over water dispersal, from South America.

These overall findings demonstrate the utility of ancient rodent DNA in eliciting individual response patterns across rodent species and exploring topics of wider evolutionary interest.

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List of Abbreviations

AD   *Anno Domini*

aDNA   ancient DNA

AMS   Accelerator Mass Spectrometry

BP   Before Present

bp   base pairs

ca.   *circa*

Cal   Calibrated

CNR   Cryptic Northern Refugia

DNA   Deoxyribonucleic acid

GI   Greenland Interstadial

GS   Greenland Stadial

Km   Kilometers

Kyr   Thousand years

LGM   Last Glacial Maximum

MIS   Marine Isotopic Stages

mtDNA   mitochondrial DNA

Myr   Million years

ORAU   Oxford Radiocarbon Accelerator Unit

PCR   Polymerase Chain Reaction

Chapter 1: General Introduction

1.1 Introduction

Evolutionary biology explores the history and processes that generate organismal diversity through a diverse set of approaches that include palaeontology, molecular biology, population genetics and biogeography. These processes are defined in terms of driving forces including mutation, genetic drift, natural selection and gene flow. In this study I have investigated evolutionary patterns and structure in the highly diverse mammalian order Rodentia, using an ancient DNA approach to more directly detect the underlying historical context.

In the following chapter I provide background detail for the study; the timeframe, the Rodentia as a group, and environmental, climatic and spatial scope, as well as general features of the methods and analyses employed.

1.2 The geologic time scale

The geologic time scale is based on layers of rock from the Earth's crust organised into standard distinguishable stratigraphic layers and calibrated into years. The International Commission on Stratigraphy aim to precisely define global units of the International Chronostratigraphic Chart to facilitate global standards for geological time scales. The time periods referred to in the subsequent chapters range from the late Mesozoic era through to the current epoch the Holocene (*Figure 1.1*).

Figure 1.1 *Schematic geologic time scale, from the Mesozoic Era to present*

Era	Period	Epoch	Time Myr
			present
Cenozoic	Quaternary	Pleistocene	0.0117 +/- 0.0013 ← Holocene Epoch
	Neogene	Pliocene	2.588 +/- 0.005
		Miocene	5.332 +/- 0.005
			23.03 +/- 0.05
	Paleogene	Oligocene	33.9 +/- 0.1
		Eocene	55.8 +/- 0.2
		Paleocene	65.5 +/- 0.3
Mesozoic	Cretaceous		
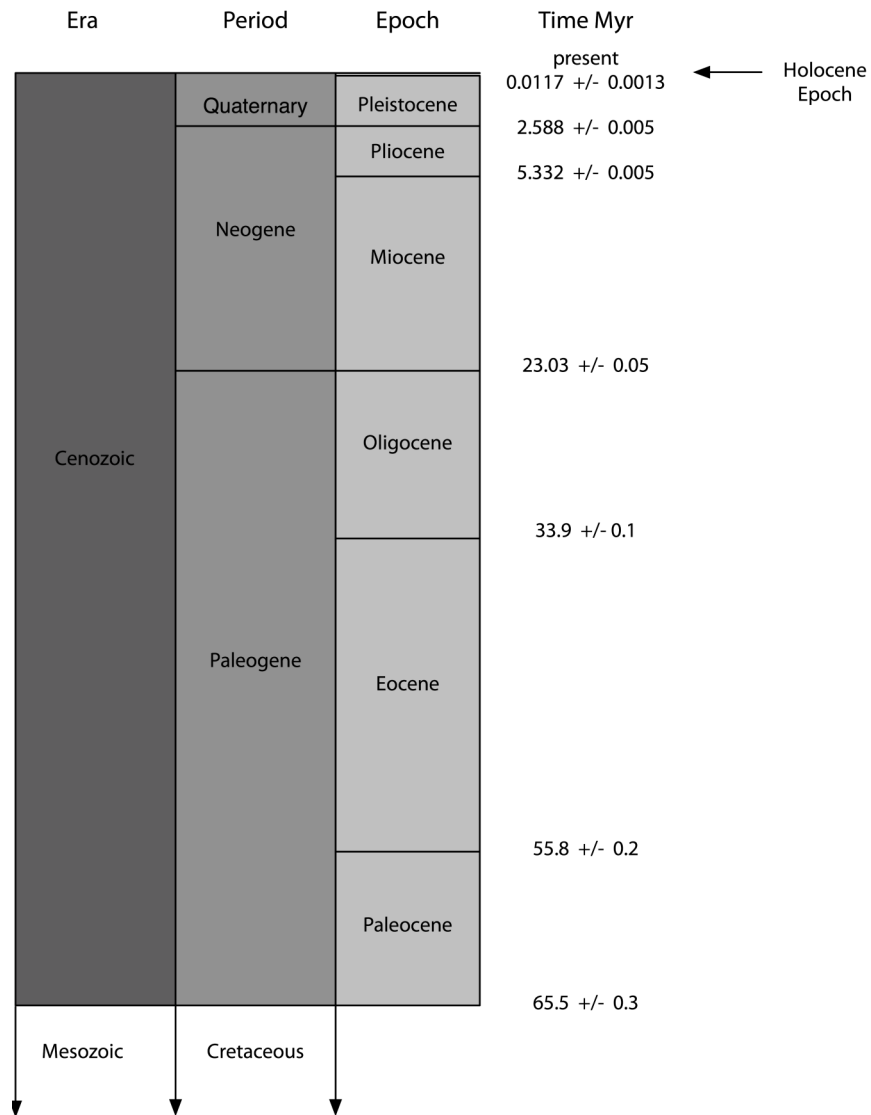
Redrawn from the International Stratigraphic Chart 2009

1.3 Rodentia

The Rodentia is the largest and most diverse order of mammals, comprising >2,100 species, the order accounts for approximately 41% of all mammal species (Gurnell, Hare, 2008). The term rodent is derived from the Latin *rodere* meaning to gnaw, a reflection on their distinctive dentition that necessitates gnawing to wear down their continuously growing incisors. The well-developed pair of elodont (open-rooted) maxillary and mandibular incisors is a defining characteristic of all rodents. Incisors are covered in thickened enamel on the

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anterior surfaces with only a thin dentine layer behind, worn down at a faster rate creating a naturally sharpened chisel-shaped edge for biting. Further characteristics include the absence of canine teeth and the presence of a diastema (gap) between the incisor and the first molar or premolar tooth, these 'cheek teeth' are anatomically indistinguishable and reduced in number to 3-5 pairs (Capello, 2008). The major taxonomic divisions among the rodentia have commonly been based on anatomical and functional differences of the complex arrangement of jaw muscles, particularly the masseters.

Rodents are ecologically highly successful, they are found in large numbers on all continents, with the exception of Antarctica, and across all habitats (except the ocean), from arid deserts to above the Arctic Circle. Rodents can be arboreal, fossorial and occasionally highly aquatic (Vaughan *et al.*, 2010). Most rodents are herbivorous, their diet includes a wide range of seeds, grasses and fruits, but others are specialist herbivores of stems and leaves or insectivores and a limited number are specialist fish eaters (Gurnell, Hare, 2008). They dominate communities in number and biomass and can be pivotal to ecosystem structure in providing an abundant food source.

Extant rodents are typically small, but in accordance with their diversity, body mass can range over four orders of magnitude, from the African Pygmy Mouse (*Mus minutoides*) weighing as little as 3.7 grams to the largest living rodent species, the South American capybara (*Hydrochoerus hydrochaeris*) that can weigh up to 81 kilograms (Millien, Bovy, 2010). The largest known rodent species however, are now extinct. Body mass estimates based on fossil evidence have been contentious and are highly dependent on the morphological variables investigated (Millien, Bovy, 2010). However, there is general agreement that *Josephoartigasia monesi* an extinct Caviomorpha rodent from the Pliocene - Pleistocene epoch found in Uruguay, is the largest recorded rodent species, its body mass was estimated to 1211 kg (+/- 753 kg) (Rinderknecht, Blanco, 2008).

The timing of the origin of rodents is a controversial issue, due to broad discrepancies between palaeontological, morphological and molecular analyses of phylogeny and the timing of divergence events (Huchon *et al.*, 2000). Fossil

evidence indicates a rodent radiation approximately 55 Myr (Hartenberger, 1998), while molecular clock estimates have consistently found an earlier Cretaceous origin. The divergence between hystricognaths and sciurognaths have been dated as far back as 75 Myr (Adkins *et al.*, 2001). That rodent diversification occurred throughout the Eocene (55-34 Myr) however, is reasonably well recorded and by 20 Myr many rodent families had emerged (Kay, Hoekstra, 2008). In general, palaeontological, morphological and molecular data concur that repeated radiations along with convergent and parallel evolution have given rise to the impressive diversity and distribution of rodents found today (Vaughan *et al.*, 2010).

The success of the rodents is often attributed to their small size and broad diet, in combination with typically r-strategist traits involving short generation times and prolific breeding - many species exhibiting increased fecundity through lactational estrus (ovulation immediately post birth) (Kay, Hoekstra, 2008). For the purpose of examining evolutionary processes, their range of ecological habitats, broad geographic distribution and abundant biomass both currently and in the past make them an ideal study system.

The near global distribution of rodents has exposed them to selective regimes from dramatically different environments and species histories. In order to gain a broad perspective whilst maintaining a level of consistency, this study has two focal regions, Europe and the Caribbean. The European studies utilise samples that span millennial timescales through periods of extensive climatic change. In contrast, the Caribbean studies analyse more recent, but highly degraded material to assess how informative this can be in understanding deeper, million year timescale events involving ancient differentiation and colonisation.

1.4 Quaternary climate change

Climate can be considered one of the most influential factors affecting organismal life. Severe climatic change leads to large-scale environmental changes and vegetational turnover. The two most immediate faunal responses to an altered and inhospitable environment are either localised extinction or migration – adaptation

would present a further, though less immediate solution. To examine rodent response to climatic change two studies focus on the Quaternary, a period associated with significant climatic and environmental change, with cycles of glacial advance and retreat throughout. The underlying cause of these global changes is thought to be external orbital forcing, a concept formalised by Milankovitch in 1941 (Milankovic, 1969). As the Earth spins around its axis and orbits the sun, the Earth undergoes cyclical changes (Milankovitch cycles) in orbital eccentricity (shape), obliquity (the angle of the Earth's axial tilt) and precession (the direction of rotation). These alter the amount of solar radiation that reach the Earth, and are enhanced or suppressed by interactions with each other. These external forces are likely further amplified by further internal factors such the extent of ice coverage and changes in green house gas concentration (Wolff *et al.*, 2010). The frequency and amplitude of climatic oscillations greatly increased through the Pleistocene, making this an ideal time frame in which to study the effects of climatic change (*Figure 1.2*).

Figure 1.2 *Pleistocene climate oscillations*
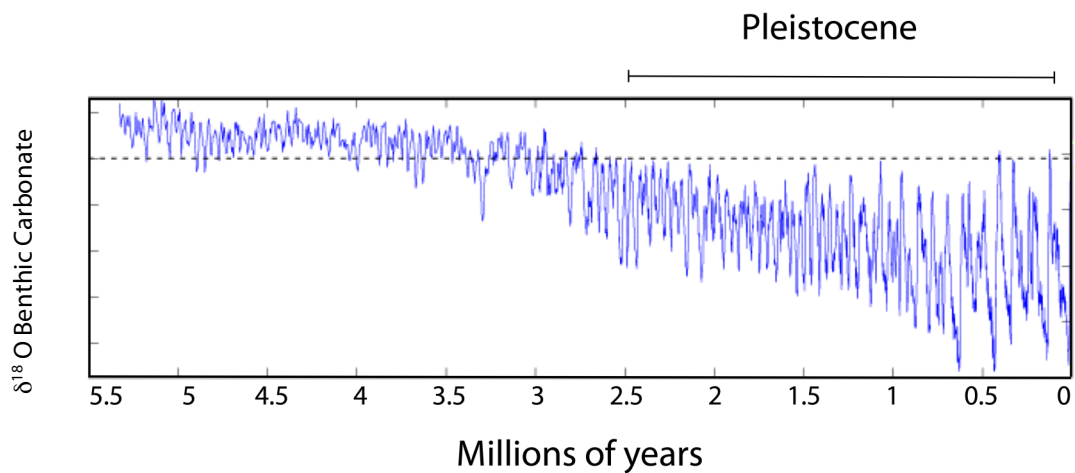
Temperature changes are represented through oxygen isotope fractionation in benthic foraminifera. The oxygen isotope ratio is used as a proxy for the mass of global ice and thus climatic change. Redrawn from (Lisiecki, Raymo, 2005)

The evidence for past global climate change over million year time scales is primarily obtained from seabed, lake bottom and polar ice cores, using carbon and oxygen isotopes and biological signatures. Deep-sea sediment cores can be used

to examine foraminifera - amoeboid protists that produce calcium carbonate shells. Over time sediments build up layers of foraminifera carbonate that can be used to measure stable isotope ratios of carbon (Shackleton et $al.$, 2004) and oxygen (Stott et $al.$, 2007). The $^{18}O/^{16}O$ ratio in calcareous foraminifera is a function of temperature and isotopic composition of ocean water in their lifetime. Oceans tend to become enriched in the heavier ^{18}O during glacial periods when the lighter ^{16}O that would preferentially be taken into the atmosphere during evaporation becomes locked within ice sheets. The $^{18}O/^{16}O$ ratio of foraminifera can therefore be used as a proxy for global ice volume (Waelbroeck et $al.$, 2002). Ice cores from Antarctica and Greenland have provided a detailed record of past climate. Air trapped in the ice can provide a direct measurement of the composition of air from the period it was trapped, while the accumulation of ice layers can be used to establish chronology. Heavier ^{18}O isotopes have a lower vapour pressure, and thus during lower temperatures they will condense faster. The $\delta^{18}O$ profile - deviation in oxygen isotope $^{18}O/^{16}O$ ratio from that of standard ocean water - therefore provides a proxy for climate (Dansgaard et $al.$, 1993). Dating the ice cores has been contentious due to reliance on ice flow modelling and error accumulation when layer counting. In the following chapters climatological dates are taken from the Greenland Ice Core Chronology 2005 (GICC05) time scale (Svensson et $al.$, 2006) based on multi parameter annual layer counting. This is one of the most recent time scales and benefits from the use of high-resolution records from the North Greenland Ice Core Project (NorthGRIP) ice core, combined with a strong accordance to other independent chronologies.

The alternating warm and cold periods derived from the oxygen isotope data taken from ice cores are referred to as marine isotope stages (MIS). In addition, stadial and interstadial periods can be detected. These are the short periods during a glacial when temperatures rise (interstadial) and short periods during interglacials when temperatures fall (stadial). The Greenland ice cores have identified 9 Greenland Interstadials (GI) in the last 40, 000 years (Svensson et $al.$, 2006). Additional periods that are frequently referred to in the following chapters are the Last Glacial Maximum (LGM) and the last - brief - cold period the Younger Dryas. The last 40,000 years are of particular significance as this is sufficiently

recent to date material through radiocarbon dating and incorporates a period of intense climatic change (*Figure 1.3*).

Figure 1.3 *Schematic of climatic oscillations during the last 40,000 years*
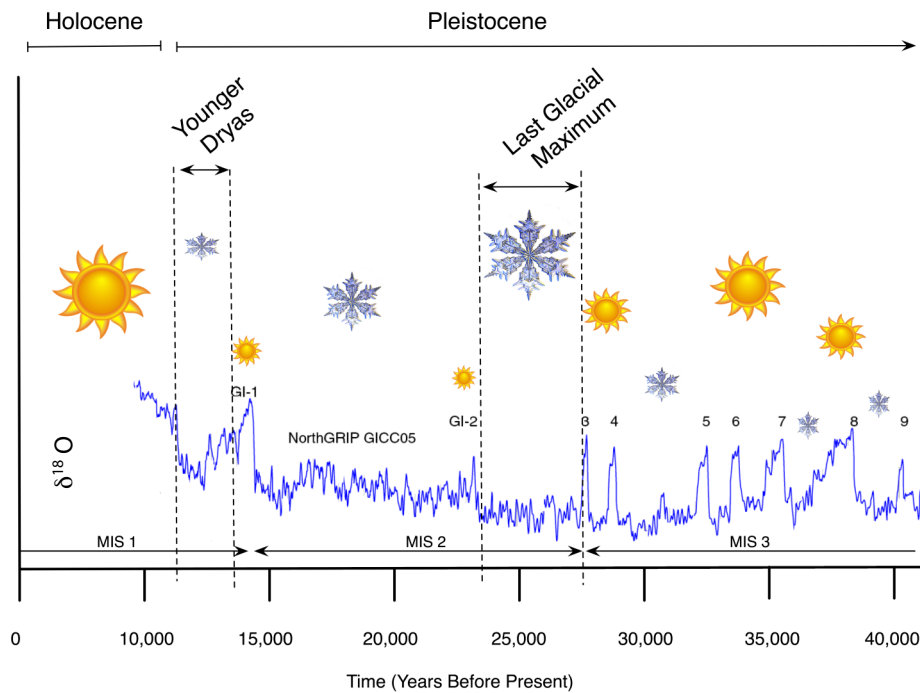
Symbols: Sun = warm period, Snowflake = cold period; the scale of the symbol represents an approximation of duration. MIS = Marine Isotope Stage. GI = Greenland Interstadial. The blue line represents temperature changes derived from oxygen isotope data from the North Greenland Ice Core Project (NorthGRIP) using the Greenland Ice Core Chronology 2005 (GICC05) time scale (Svensson et al. 2006)

1.5 Europe

Europe provides an ideal locale to study the effects of Quaternary climate change on rodent species. During interglacials Europe is a temperate zone inhabited by a range of small temperate mammal species. During glacial periods, ice sheets spread down from the north, leaving Northern Europe almost fully glaciated and permafrost extending throughout Central Europe, essentially leaving only the southernmost peninsular ice free (Svendsen *et al.*, 2004) (*Figure 1.4*).

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Figure 1.4 *The extent of ice, permafrost coverage during the Last Glacial Maximum (LGM) ca. 27 – 23 Kyr BP*
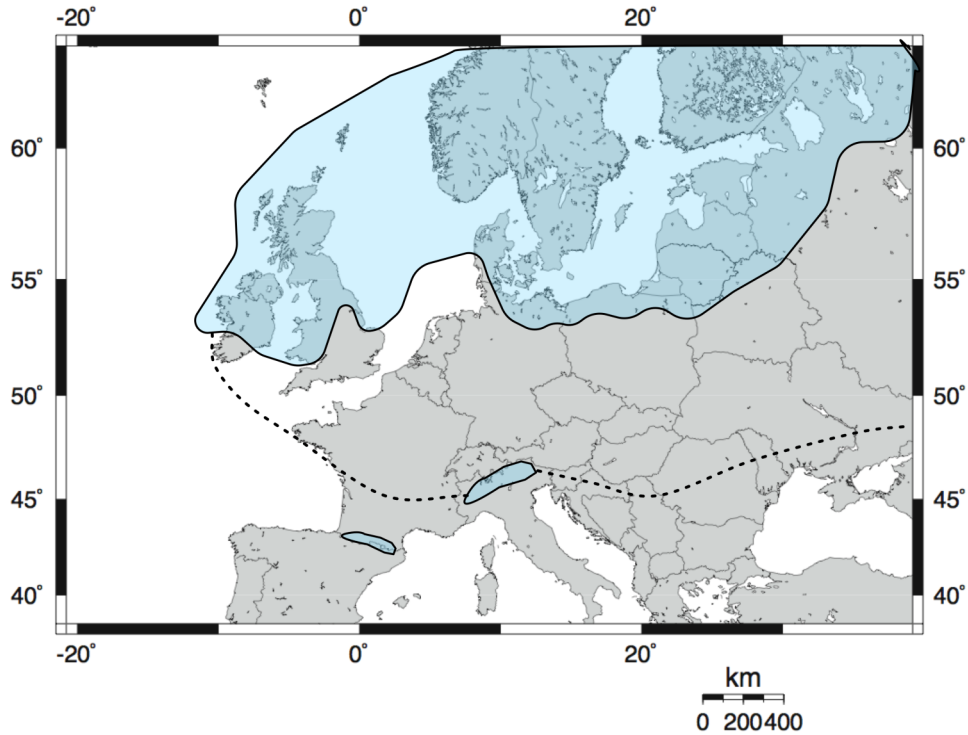
The solid dark lines represent the extent of ice coverage, dotted lines the extent of permafrost during the LGM. Redrawn from (Svendsen et al., 2004; Willis, 1996)

Temperate rodent species can therefore inhabit a European wide range during interglacials that during glacials would be severely restricted to southern peninsula (Hewitt, 1996) or refugia (Stewart, Lister, 2001). The converse is true for Artic species, currently restricted outside of Europe to the Artic tundra. During glacial periods their preferential habitat expands south resulting in Arctic tundra species throughout Northern and Central Europe. In order to appreciate individual responses to these opposing circumstances I investigate an Arctic species, the arctic collared lemming (*Dicrostonyx torquatus*) and a temperate species, the water vole (*Arvicola terrestris*) in Northern Europe from the time of the LGM through the end of the Pleistocene and into the early Holocene.

1.6 Caribbean

The second region to be explored - chapters 4 and 5 - is the Caribbean, specifically the Greater and Lesser Antillean islands. (*Figure 1.5*)

Figure 1.5 *Map of the Caribbean Region*
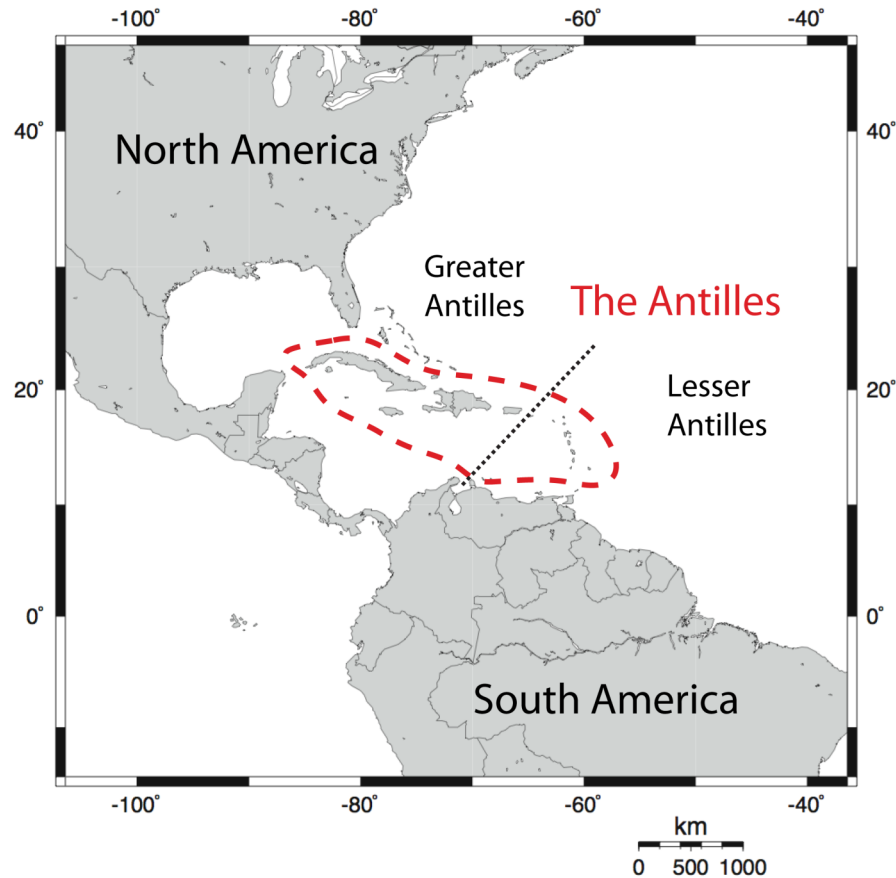
The dashed red line indicates the Antillean islands; these are comprised of the Greater Antilles to the west and the Lesser Antilles to the east

The Antilles is a fascinating region in which to study rodent evolution, in part due to the complex geological history of the islands. It is generally accepted that initiation of the Antillean island arc system began with the formation of the Caribbean Plate during the Cretaceous, ca. 145 - 65 Myr. Upon formation, the Caribbean Plate is thought to have moved in an east-northeast direction. During this movement the heavier North American Plate moved under the Caribbean

Plate, with the resulting subduction and volcanism creating a proto-Antillean arc (Burke, 1988). Collision with the Bahamas Platform caused the subduction to cease, fixing Cuba, northern Hispaniola and Puerto Rico to the American Plate. The eastern edge of the Caribbean Plate continued to move, initially forming the Aves Ridge, but with further eastward movement the Lesser Antilles. During this period Cuba, northern Hispaniola and Puerto Rico separated from the American Plate, while Jamaica and southern Hispaniola had remained attached to the Caribbean Plate moving east. Jamaica remained in an isolated position, but southern Hispaniola collided with northern Hispaniola during the middle to late Miocene (Hedges, 2006).

There is general agreement that since the Middle Eocene (< 40 Myr) there have been permanent subaerial (above water) landmasses - the (larger) Antillean islands - across the West Indies. However, debate continues with regards whether landmasses remained permanently above sea level between the formation of the Antilles in the Cretaceous and the Eocene (Hedges, 2006; Iturralde-Vincent, MacPhee, 1999; MacPhee, Iturralde-Vincent, 2005). Evidence from marine sediments is incomplete, but suggestive that the Cretaceous proto-Antillean landmass was subaerial, although not necessarily as a single landmass (Hedges, 2006). The most extreme proposes that until 37 Myr ago there were no persistent subaerial landmasses in the Antilles, only limited periods of emergence, thus the current biota of the Antilles can only reflect colonisation events after this period (Hedges, 2006).

The complexity of the geological history of the Antilles has led to several competing although not necessarily mutually exclusive proposals for the origins of faunal colonisation. Prior to the 1960s - and the development of plate tectonic theory - overwater dispersal was the only viable hypothesis for explaining the presence of land mammals on oceanic islands (Morgan, Woods, 1986). However, as an understanding of the transience of plate systems developed, vicariant models became more popular; with regards to the Antilles two main vicariant models have been widely discussed. The Cretaceous origins of the Antilles as a proto-Antillean archipelago has led to the theory that the landmass was connected to North and South America, allowing free movement of fauna onto the archipelago.

When the three landmasses separated during the Oligocene (ca. 30 Myr), the Antillean landmass carried with it ancestral fauna and flora from North and South America (Rosen, 1975; 1985). A second model proposes a land bridge, connecting the Greater Antilles and northwestern South America through the Aves Ridge; the Greater Antillean and Aves Ridge hypothesis (GAARlandia hypothesis), (Iturralde-Vincent, MacPhee, 1999; MacPhee, Iturralde-Vincent, 2005). The landspan is proposed to have been subaerial, (though the authors do not commit to whether this is continuously subaerial) around the Oligocene/Eocene transition (35-33 Myr) lasting for approximately 1 Myr.

Vicariant models therefore require a specific timeline for species colonisation events; the proto-Antillean archipelago requires diversification of island species from continental species to date around the time of the Cretaceous, while a narrow window of opportunity exists for the GAARlandia hypothesis (ca. 35-33 Myr). It therefore follows that if either of these models were exclusively correct, estimates of when species diverged from the mainland (either through molecular or fossil dating) would cluster around these time points. Divergence dates however, vary considerably across taxa, some report a Mesozoic origin (Roca *et al.*, 2004), a few are compatible with the GAARlandia landspan 35-33 Myr (MacPhee *et al.*, 2000) and several are spread throughout the Cenozoic, occurring as recently as ca. 5Myr (Dávalos, Turvey, in press). This certainly negates either model as an exclusive mode for Caribbean colonisation but does not disprove over land colonisation *per se*, instead suggesting a more complex scenario with individual species considered on a case-by-case basis.

The other more traditional model for faunal colonisation is via overwater dispersal. For volant species such as birds and bats this does not pose a significant problem, but terrestrial mammals require a form of transportation. Mats of vegetation and flotsam washed out from river mouths and into ocean currents are considered the most likely methods for a terrestrial species to 'raft' from a mainland to an oceanic island (Hedges, 2001). The orientation and flow of ocean currents therefore have a significant role in dispersal patterns of mainland to island species translocation. In the West Indies the predominant current flows from the southeast to the southwest due to the clockwise flow of the Atlantic in

this region. Water currents flow from the west coast of Africa across to the northeast of South America, moving across the Caribbean east to west to the tip of Florida, to form the Gulf Stream (Hedges, 2001). Since the origin of the Caribbean Plate, the West Indies have remained north of the equator, past currents would therefore also have flowed in the same general clockwise pattern (Hedges, 1996). This implies that if colonisation of the Lesser Antilles were a result of overwater dispersal South America, rather than North America, would be the mainland source of origin. An additional natural conveyor in the region are frequent hurricanes and storms that have also been postulated as aiding overwater dispersal (Calsbeek, Smith, 2003).

Further support for the overwater colonisation of the West Indies is derived from species composition. Across the islands both current and fossil evidence demonstrate a depauperate suite of higher-level taxa. There is no evidence of carnivores, lagomorphs or marsupials ever having occurred on the islands and many families of frogs and snakes are also absent (Hedges, 2006). This disproportionate reflection of mainland species is thought to be more indicative of islands that were colonised by the 'random' nature of overwater dispersal, as opposed to colonisation via a land connection that would result in a fuller representation of mainland species diversity (Williams, 1989).

In order to investigate how the geological complexity of this region influenced rodent evolution including ancient differentiation and colonisation, I examine one of the few remaining extant endemic species, the Hispaniolan hutia (*Plagiodontia aedium*) and a group of extinct Antillean rice rats (Oryyzomyini).

1.7 Ancient DNA

Genetic data from modern populations have been revolutionary in understanding population dynamics such as abundance and distribution and can be highly effective in examining recent events such as human induced population decline (Olivieri *et al.*, 2008) and hybrid zones (Haas *et al.*, 2009). Population history relating to events from the more distant past however, are not always apparent or accurately definable from analysis of modern genetic data alone. Genetic

signatures can be rendered undetectable or misleading, due to population shifts in size or gene flow, that mask prior genetic structure through the reestablishment of genetic equilibrium when modern samples are examined in isolation (Ramakrishnan, Hadly, 2009).

The application of an ancient DNA approach can bridge this hiatus in knowledge by providing a direct view of past genetic structure. Ancient DNA (aDNA) techniques have been applied to examine a number of species involving a variety of evolutionary questions. These include the exploration of extinct species and their relationship to extant taxa. The extinct Australian thylacine (*Thylacinus cynocephalus*) showed many morphological and ecological similarities to the Northern Hemisphere Canidae family (wolves and dogs), however ancient DNA analyses have shown that they hold a basal position in Dasyuromorphia - an order that comprises most carnivorous marsupials – and are therefore an example of convergent evolution (Miller *et al.*, 2009). The New Zealand moa (*Dinornis*) thought to represent at least three size differentiated species were found to represent a single species exhibiting extreme reversed sexual size dimorphism (Bunce *et al.*, 2003).

In addition, aDNA has provided new insights into the location and timing of animal domestication events, which in turn can act as a proxy for associated human expansion and migration. Traditional archeological evidence had previously suggested that pig (*Sus scrofa*) domestication had originated from limited domestication events in the Near and Far East. The application of an aDNA approach has highlighted a more complex scenario suggesting multiple domestication centres across Eurasia through the identification of regional signatures from wild populations reflected in modern domestic samples (Larson *et al.*, 2005). Further patterns of regionally distinct wild boar and domesticated pigs have also been found throughout Eastern Asia (Larson *et al.*, 2010).

The use of aDNA in reconstructing past histories have also highlighted population level movements and extinctions that would otherwise have gone un-detected. Extant brown bear (*Ursus arctos*) populations exhibit geographically distinct lineages, thought to represent low dispersal rates and barriers to gene flow.

Ancient DNA analyses however, found that whilst a strong genetic structure was maintained over long periods of time, Beringian brown bears experienced several phases of population dispersal, extinction and recolonisation (Barnes *et al.*, 2002). In contrast, contemporary Beringian bison (*Bison priscus*) populations went into steep decline, their genetic diversity fluctuating during periods of climatic change (Shapiro *et al.*, 2004). Studies such as these have proved highly effective in utilising aDNA to assess population histories and the varied responses to environmental change.

The larger mammal fauna, has to date been the most thoroughly researched; in contrast much less aDNA work has been conducted on small mammals. One of the first studies to examine rodent aDNA utilised museum specimens of the Panamint kangaroo rat (*Dipodomys panamintinus*) to analyse genetic diversity over a range of geographically distinct subspecies through time (Thomas *et al.*, 1990). Rodents were also the focus for one of the first studies to employ a phylochronological approach, analysis of a single location through time (Hadly *et al.*, 2004). The study focused on the northern pocket gopher (*Thomomys talpoides*) and the montane vole (*Microtus montanus*) from continuous fossil deposits spanning 3,000 years. Abundance of fossils indicated that both species had suffered severe decline in numbers. Analysis of the aDNA revealed that the montane vole showed increased levels of diversity that modelling indicated could only have occurred through immigration. Therefore, a simple decline in numbers was actually masking a more complex scenario of migration with expansion and decline of individual populations/genotypes. The study also highlighted that similar ecological responses – climatic change likely led to the decline in both gopher and vole abundance – did not constitute similar genetic response – no evidence of increased diversity/immigration were detected in the pocket gopher population. A further aDNA study found steep decline in genetic diversity of the South American tuco-tuco (*Ctenomys sociabilis*) in association with palaeoenvironmental change during the Holocene (Chan *et al.*, 2005).

1.8 Difficulties associated with an ancient DNA approach

1.8.1 Small sample sizes

The inclusion of ancient DNA has the potential to be highly informative; its application however, can be problematic. Suboptimal sample sizes and inability to obtain material from a particular time or location are a common issue. This can be due to limited sample availability and/or inaccessibility, *i.e.* permission to destructively sample is denied. When the species under investigation is also physically small, such as with rodents, this poses additional problems. Species level identifications are often obtained through tooth morphology and in the absence of a complete skeleton – that in itself would be small – a mandible may be the only accurately identified material available. Not only does this require permission to destroy what is potentially the entirety of the identified individual, it also means that the total amount of material per individual is highly constrained. This places additional pressure on extraction and purification processes and the potential to maintain sufficient material to pursue further analyses such as radiocarbon dating.

1.8.2 aDNA degradation

A major source of concern with regards aDNA studies has been the reliability of aDNA amplification. A living cell maintains the integrity of DNA through repair and protection, but from the moment an organism ceases to live these energy dependant processes also cease. Cellular enzymes such as lysosomal nucleases, sequestered in living cells, upon death can access and degrade DNA along with microbes and insects that feed on decaying matter (Pääbo *et al.*, 2004). *Post mortem* DNA damage physically destroys the molecule thus reducing the length and number of amplifiable DNA molecules. Chemical reactions can also lead to DNA modifications that can either block the extension of polymerase enzymes or permit amplification but result in miscoding lesions - erroneous base incorporation during the polymerase chain reaction (PCR) (Pääbo, 1989). Miscoding lesions can be caused by regular PCR polymerase amplification errors or through the amplification of biochemically damaged template molecules. Two types of spontaneous chemical reactions commonly affect *post mortem* DNA, oxidative and hydrolytic damage. Oxidation causes base fragmentation and

damages deoxyribose residues resulting in breakage of the DNA sugar-phosphate backbone. This can result in complete strand breakage or nucleotide modifications. Hydrolytic damage can result in deamination of bases and depurination and depyrimidination (Höss *et al.*, 1996). Both oxidative and hydrolytic damage can therefore lead to base modifications that result in blocking or miscoding lesions. In aDNA commonly observed miscoding lesions are transitions, adenine → guanine (A → G), cytosine → thymine (C → T), G → A and T → C (Gilbert *et al.*, 2003). These are often grouped into type 1 transitions (A → G/ T → C) and type 2 transitions (C → T/ G → A). These are caused by the deamination of adenine to hypoxanthine and the deamination of cytosine to uracil, respectively. Type 2 miscoding lesions (caused by the deamination of cytosine to uracil) are thought to represent the majority of damage derived errors in aDNA (Gilbert *et al.*, 2007).

The rate of degradation is highly dependent on the environment and subsequent storage conditions. Constant low temperature and freezing are thought to be of greatest importance in reducing the rate of *post mortem* degradation in DNA (Willerslev, Cooper, 2005). Frozen and permafrost deposits from arctic and subantartic regions have proved advantageous for the long-term survival. However, cold cave deposits from Southern Chile have also yielded well preserved samples (Höss *et al.*, 1996). Warm regions have generally been associated with a faster rate of DNA degradation, and preservation of retrievable aDNA has been estimated to the region of a few thousand years (Gilbert *et al.*, 2005b; Poinar *et al.*, 1996). As a result very few studies have been published on the recovery of ancient DNA from a warmer climate, notable exceptions include a Neandertal skeleton from Northern Spain (Lalueza-Fox *et al.*, 2005b), a subfossil bone from a Balearic bovid (*Myotragus balearicus*) (Lalueza-Fox *et al.*, 2005a), nine subfossil samples of giant lemur (*Palaeopropithecus* and *Megaladapis*) from Madagascar (Karanth *et al.*, 2005), pigs (*Sus*) from Southeast Asia and Oceania (Larson *et al.* 2007) and three museum specimens of the Cuban solenodon (*Solenodon cubanus*) (Roca *et al.*, 2004).

1.8.3 aDNA contamination

A further obstacle to the successful amplification of authentic aDNA is the issue of contamination. The hazards involved with ancient human DNA are immediately apparent, however, all aDNA experiments are at risk of contaminants from successful PCR products. PCR reactions create large concentrations of products that can remain stable over long periods of time, once aerosolized they can easily spread throughout a laboratory (Greenwood, 2009). Transportation of PCR product droplets can be via an individual's skin, clothing or any object that has been in the post PCR vicinity. These microscopic droplets have the potential to contain a thousand times the amplifiable DNA present in an ancient DNA sample (Willerslev, Cooper, 2005). Thus if a microscopic amount of PCR product is present during any part of the pre PCR procedure this could easily be preferentially incorporated as a template molecule and produce erroneous DNA results. Further sources of contamination can arise from laboratory reagents, equipment and the sample itself, all of which may have been exposed to exogenous DNA from the environment, including bacteria and general pest species such as mice (*Mus*) - a common presence in museum and storage facilities.

1.9 Resolutions and precautionary measures

1.9.1 Small sample sizes

Reducing the amount of material required in the initial extraction process may prove advantageous when attempting to gain permission to destructively sample material. It also has potential to maintain sufficient material for additional analyses such as radiocarbon dating.

1.9.2 aDNA authentication

To ensure the validity of ancient DNA both as a non-contaminant and accurately coded (undamaged) sequence, a number of precautions are now taken as standard when working with ancient DNA (Cooper, Poinar, 2000; Gilbert *et al.*, 2005a; Pääbo, 1989). Great importance is placed on the physical separation of pre and post PCR laboratories with a strict policy of no daily personnel movement from the post PCR to the pre PCR laboratory. The pre PCR laboratory is dedicated to

ancient DNA research i.e. no modern material are analysed in that area. Extensive cleaning of the laboratory with dilute sodium hypochlorite and the exposure of equipment and reagents to ultra violet (UV) irradiation are carried out daily.

Extraction processes aim to preserve DNA structure while purifying the sample of PCR inhibitors. The methods employed in the following chapters utilise an initial digestion of samples with ethylenediaminetetraacetic acid (EDTA- to decalcify the bone), protinease K (to lyse cells and release the DNA) and urea (a protein solubilising agent). The DNA is then purified directly using silica-based spin columns ensuring a minimalist approach, found to be more efficient and to reduce the risk of contamination (Rohland, Hofreiter, 2007; Yang *et al.*, 1998). In addition, monitoring for potential contaminants is maintained through the use of positive and negative controls throughout extraction and PCR processes.

Targeted regions of DNA are amplified through the process of PCR. When amplifying aDNA a crucial step is in primer design. Short fragments of DNA have been shown to possess a greater chance of survival (Smith *et al.*, 2003), thus primer pairs typically target approximately a 150 base pair region, increasing the likelihood of obtaining non-damaged DNA. The primer pairs are nested to ensure that each amplified fragment overlaps, thus contaminant DNA and miscoding lesions are more easily identifiable. Positive results obtained are regularly repeated to check for authenticity and all results are considered in terms of whether they make phylogenetic sense.

1.10 The use of mitochondrial DNA (mtDNA)

MtDNA has been extensively used in aDNA analyses. One of the main advantages is that of high copy number, there are thousands of mitochondria in every cell. The mtDNA is also protected by a two-walled organelle and the circular shape of the molecule helps prevent exonuclease degradation, these features increase the probability of obtaining non-degraded DNA (Pereira *et al.*, 2010). MtDNA in animals is typically transmitted uniparentally through female inheritance, thus lacks recombination. This benefits genetic analysis as haplotypes can be transmitted across many generations, and new lineages can only arise

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through mutation, the frequency of which is predominantly controlled by migration and drift.

Animal mtDNA is a small molecule - typically 14 to 20kbp - and contains 37 genes essential for normal mitochondria function resulting in energy production (Ballard, Whitlock, 2004). Regions are therefore mainly well conserved with only limited areas that are non-coding. Regions can therefore be targeted dependent on the type of analysis being undertaken. The regions used in the following chapters are the cytochrome b oxidase (cyt*b*) and the control region. The cyt*b* gene is commonly used for deeper phylogenetic relationships and congenera divergence as it is a relatively well-conserved region, but can contain sufficient variation to allow analysis of population level differences (Johns, Avise, 1998). This is thought to be due to the slow evolution of non-synonymous substitutions and relatively fast evolution of silent positions (Irwin *et al.*, 1991). The control region has a highly conserved domain flanked by hypervariable regions, making this an ideal region to examine within species differentiation in the not too distant past (Avise, 2000). When investigating older lineages, identification can be confounded by site saturation – back mutations – due to the higher mutation rate of the control region.

1.11 Analyses

Phylogenetic studies allow the reconstruction of evolutionary relationships between taxa. This is based on the assertion that all life is related by common descent and that closely related taxa will have shared a common ancestor more recently than distantly related taxa. Phylogenetics attempts to reconstruct evolutionary histories through the similarities and differences in DNA sequence data. In the following chapters I employ two methods to construct phylogenies from ancient and historical DNA, maximum likelihood and Bayesian.

1.11.1 Nucleotide sequence evolution
In order to utilise molecular data to establish evolutionary relationships, maximum likelihood and Bayesian methods require a suitable model of nucleotide sequence evolution. Nucleotide substitution can be modelled as the probability of

change from one state to another (one nucleotide to another) a value dependent on the time that has passed and the rate at which substitutions occur. Nucleotide changes can involve insertion and deletion events (indels) or point mutations. Point mutations can be transitions – purine to purine (A ↔ G) or pyrimidine to pyrimidine (C ↔ T) and transversions - purine to pyrimidine and vice versa, transitional changes being more frequently observed (Kimura, 1980). Models of nucleotide evolution need to take into account that as genetic distance increases sites can undergo multiple substitutions and observed distances may require some correction to control for this.

In the following chapters I apply the program Modeltest (Posada, Crandall, 1998) to select the appropriate nucleotide evolutionary model to fit the data under investigation. Modeltest provides likelihood scores for 56 different models of DNA substitution, all of which are nested within the General Time Reversible (GTR) model. Models differ in their free - adjustable - parameters. Increasing the number of free parameters can better approximate the complex reality of evolution and will increase the fit between the model and the data. However, by increasing free parameters this increases the variance and reduces the power to discriminate so the model can 'over-fit' the data. The addition of a parameter therefore needs to make a significant improvement for it to be of value. This can be tested using hierarchical likelihood ratio tests (hLRT), where pairwise comparisons between nested models are repeated, until the simplest model at a given significance level has been reached. An alternative measure of fit is the Akaike Information Criterion (AIC). This method scores the fit of the model to the data – log likelihood - but adds a penalty for additional parameters. The AIC scores simultaneously, whereas the hLRT scores hierarchically, the order of the hLRT can therefore affect the model selected. However, studies have illustrated that differences between the two rarely affect evolutionary inferences and that any use of statistical based model selection is preferable to omitting the process (Ripplinger, Sullivan, 2008).

Typical nucleotide substitution models are illustrated in *Figure 1.6*, those utilised in the following chapters are:

Kimura 2-parameter – Assumes that all nucleotides occur with the same frequency, but transitions and transversions can evolve at different rates.

Hasegawa-Kishino-Yano – Assumes that different nucleotides can each have a different frequency. Transition and transversion rates can also be variable.

Tamura-Nei – Assumes variable base and transition transversion frequencies, but also permits the two types of transitions (A → G and C → T) to have different rates.

GTR – Assumes variable base frequencies and a symmetric substitution model. This incorporates a time reversible element as A changes to T at the same rate that T changes to A. Each pair of nucleotide substitutions can evolve at a different rate.

Figure 1.6 *Schematic for typical nucleotide substitution models*
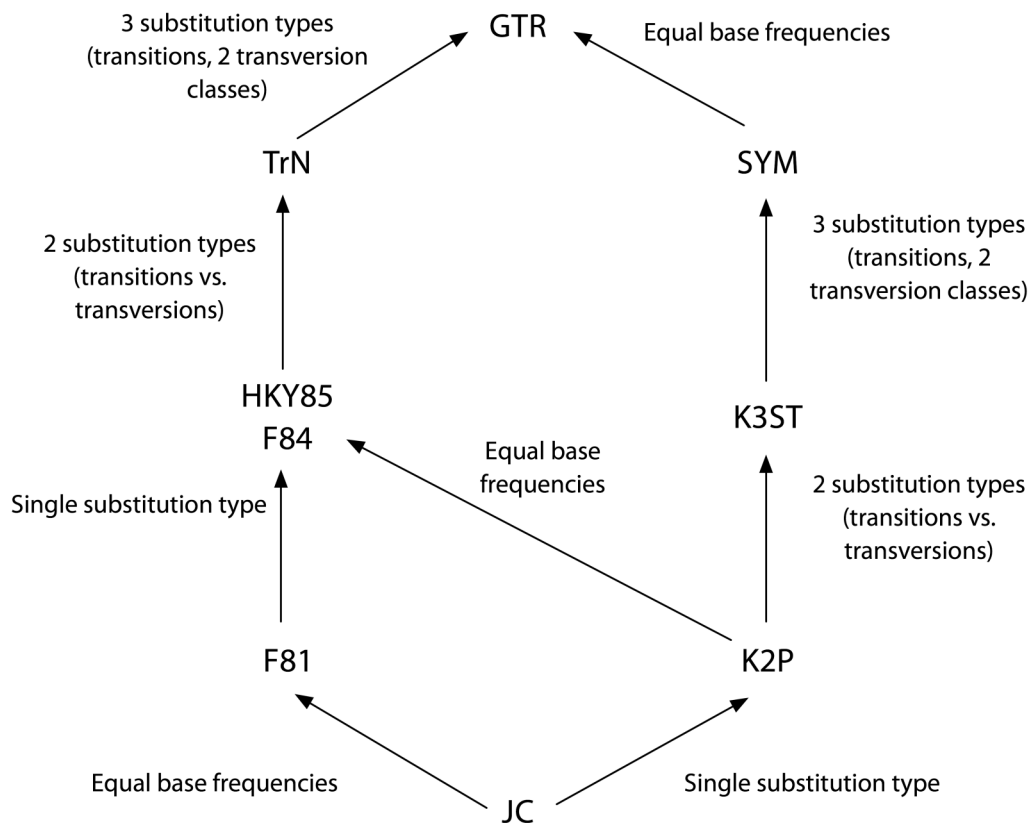
Abbreviations: GTR, General Time Reversible (Lanave et al., 1984); SYM (Zharkikh, 1994); K3ST (Kimura, 1981) three substitution types; K2P (Kimura, 1980) two-parameter model; JC (Jukes, Cantor, 1969); F81 (Felsenstein, 1981); F84 (Felsenstein 1993); HKY85 (Hasegawa et al., 1985); TrN (Tamura, Nei, 1993). Figure was redrawn from Swofford et al. (1996)

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In addition to the rate of nucleotide change, further model parameters can be implemented to describe the variation in rates among sites in a sequence. Models that have been used in the following chapters are:

Gamma distribution – The distribution has a shape parameter, α, that is inversely proportional to the amount of among-site rate differences – a low value indicates that proportionately few sites are experiencing the majority of substitutions.

Proportion of invariable sites – an estimate of the proportion of unchanging sites.

1.11.2 Maximum likelihood

In essence, likelihood is the probability of generating the dataset (the sequence data) given a particular model (tree topology and sequence evolution model). Within the sequence data each nucleotide site has a likelihood value, the total log likelihood is the sum of the log of the site likelihoods, and the maximum likelihood tree is the tree topology that provides the highest likelihood under the given model (Hall, 2005). A maximum likelihood approach therefore requires a specified model of nucleotide sequence evolution as described above. In the following chapters I employ PAUP* (Swofford, 2000) to construct an initial tree (from the sequence data provided), and an evolutionary model with optimised parameters fixed according to the simplest model that best fits the data - estimated in Modeltest. A heuristic search utilises a branch-swapping algorithm, tree-bisection-reconnection (TBR) to evaluate branch rearrangements (branch order and branch length) from the initial tree. The tree with the highest likelihood is the best estimate of the true phylogeny.

1.11.3 Bootstrapping

Bootstrapping (Felsenstein, 1985) permits an evaluation of the phylogeny constructed through testing whether the data obtained reflect all possible data sufficiently to give confidence in the estimated tree. The bootstrap technique samples data points (nucleotide characters) from the sequence data one at a time, replacing them in the datapool and resampling. The process creates a new data set where some data (nucleotides) are represented more than once, others not at all. The tree is then reconstructed from this dataset and topology compared to the original tree. Interior branches of the original tree are scored as either 1 or 0 depending on if they are the identical (1) or different (0) from the resampled tree.

The procedure is repeated several hundred times, the final bootstrap value for a branch is the proportion of samples that it has been allocated a value of 1 (Felsenstein, 1985).

1.11.4 Bayesian

The Bayesian approach to phylogeny construction also implements a likelihood function, but in this method it is the probability of the model (tree topology and sequence evolution model) given the data (sequence data). The free parameters optimised in the ML approach are sampled probability distributions under the Bayesian approach. The goal is to quantify a full posterior probability distribution over the entire range of possible tree topologies and model parameter values. This goal is too complex to solve analytically, it is therefore achieved through an approximate solution by sampling in parameter space using a Metropolis-coupled Markov chain Monte Carlo (MCMCMC) approach. In this process several chains are run in parallel with multiple 'heated' chains moving more rapidly to cover more parameter space (Metropolis-coupled). Heated chains can swap with the single cold chain, but only the cold chain is actually sampled. All chains take a random 'walk' through tree and parameter space, sampling values in proportion to their probability distribution (Monte Carlo). Random trees and parameters are therefore sampled, then either rejected or accepted depending on the value of the posterior probability (Markov process), higher values are always accepted and although lower values can be accepted, small steps downwards are accepted more often than large ones (Kelly, 2005). Typically a chain starts with a burn in period, the results of which are ignored. After the burn in period the chain should have reached a plateau - a plot of the likelihood of the chain can be observed to assess convergence – samples from the plateau can be used to construct the posterior distribution of all the parameters and trees. Trees are sampled at fixed intervals and the posterior probability of the tree is approximated by the proportion of times that it was visited by the cold chain (Yang, Rannala, 1997). The sampled trees can also be used to construct a consensus tree. The consensus indices - the proportion of sampled trees that contain an individual partition - provide Bayesian posterior probability estimates of clade credibility.

1.11.5 Divergence dating

Divergence dating also stems from the concept of common descent. It centres on the theory that differences in DNA sequence data are a function of time since evolutionary divergence. This was initially proposed by Zuckerkandl and Pauling (1965) using protein sequences of hemoglobins, amino acid substitution rates and fossil calibration dates. In general, this is known as the molecular clock hypothesis, whereby DNA evolves at roughly constant rates over time and across evolutionary lineages. The implication being that if genetic divergence accumulates in a clock like manner, differentiation can be utilised to estimate the timing of past evolutionary events. Initial theories advocated 'universal' clocks, mtDNA of mammals was thought to universally evolve at approximately 2% sequence divergence per million years (Brown *et al.*, 1982). It has since been appreciated that the rate of evolutionary change across taxonomic groups and regions of DNA under investigation are more varied and complex (Li *et al.*, 1987). However, 'local' clocks for specific DNA regions can be applied within groups that share similar metabolic rates, generation times and DNA repair efficiency (Yoder, Yang, 2000)

As with phylogenetic tree construction a suitable model of nucleotide evolution needs to be employed. This facilitates a more reliable estimate of actual sequence divergence through incorporating known parameters affecting nucleotide substitution rates that increase sequence saturation over time. The goal is to obtain a correct estimation of the average number of substitutions per site (branch length). In the following chapters I employ BEAST (Drummond, Rambaut, 2007) a Bayesian statistical framework that utilises posterior probability distribution of parameters given a set of molecular sequences and using the MCMCMC algorithm, in much the same way as previously described for phylogeny construction. In addition, the rate of evolution is explicitly modelled for each branch of the tree, thus a known or estimated mutation rate can be incorporated into the model and used to estimate divergence points on the tree.

1.11.6 Coalescent modelling

Coalescent theory involves a retrospective model that traces populations backwards in time to a common ancestral point, the most recent common ancestor (MRCA). As with molecular clock analysis, emphasis is placed on population divergence to estimate the time to the MRCA, the time of coalescence. It can be used to explore the coalescent time – number of generations since MRCA- and theta – a product of the relationship between effective population size and mutation rate. A population with high diversity will take longer to reach coalescence than a population with low diversity (Sigwart, 2009). Coalescent modelling has therefore provided an effective tool for examining evolutionary processes and demographic events. Modelling programmes such as Serial SimCoal (SSC) (Anderson *et al.*, 2005) can now be employed to simulate genetic data under increasingly complex demographic conditions whilst estimating further defined parameters of interest. Thousands of trees can be simulated using a combination of parameters that are either defined directly, when the value is a known, such as generation time or provided as a range of plausible values under exploration. A comparison of the summary statistics derived from each of the simulated trees against summary statistics from the 'real' sequence data illustrate which of the histories or parameter values have the highest probability of having generated the original data. This method can be particularly effective in hypothesis testing, where a plausible demographic history such as a population bottleneck (Campos *et al.*, in press) or migration event (Valdiosera *et al.*, 2008) are suspected.

1.12 Thesis aims

1.12.1 (Aim A) To assess the utility of rodent aDNA analyses

A1: Determine if sample masses are sufficient to permit the generation of authentic DNA sequence data.

A2: Determine if the amount of material required for extraction and purification procedures can be further reduced to permit additional analyses such as radiocarbon dating.

A3: Determine if it is possible to extract authentic DNA sequence data from small sample masses where the material may be highly degraded material - both through time and warm climate.

1.12.2 (Aim B) To identify any consistent patterns across rodent species

B1: In response to climate change

B2: With reference to genetic diversity

1.12.3 (Aim C) To review the merits of working with rodent aDNA

C1: With regards to data that would otherwise have been obscured or irretrievable

C2: Concerning broader topics of general interest/concern

1.13 Thesis studies

To achieve these aims I have investigated four groups of rodents, arctic collared lemmings, water vole, hutia and rice rats, focusing on two distinct regions, Europe and the Caribbean. The following four chapters explore patterns and structure within these diverse species histories, examining the effects of dramatically different environments.

1.14 References

Adkins RM, Gelke EL, Rowe D, Honeycutt RL (2001) Molecular Phylogeny and Divergence Time Estimates for Major Rodent Groups: Evidence from Multiple Genes. *Molecular Biology and Evolution* **18**, 777-791.

Anderson CNK, Ramakrishnan U, Chan YL, Hadly EA (2005) Serial SimCoal: A population genetics model for data from multiple populations and points in time. *Bioinformatics* **21**, 1733-1734.

Avise JC (2000) *Phylogeography: The history and formation of species* Harvard university press.

Ballard JWO, Whitlock MC (2004) The incomplete natural history of mitochondria. *Molecular Ecology* **13**, 729-744.

Barnes I, Matheus P, Shapiro B, Jensen D, Cooper A (2002) Dynamics of Pleistocene Population Extinctions in Beringian Brown Bears. *Science* **295**, 2267-2270.

Brown WM, Pragner EM, Wang A, Wilson AC (1982) Mitochondrial DNA sequence of primates: Tempo and mode of evolution. *Journal of Molecular Evolution* **18**, 225-239.

Bunce M, Worthy TH, Ford T*, et al.* (2003) Extreme reversed sexual size dimorphism in the extinct New Zealand moa Dinornis. *Nature* **425**, 172-175.

Burke K (1988) Tectonic Evolution of the Caribbean. *Annual Review of Earth and Planetary Sciences* **16**, 201-230.

Calsbeek R, Smith TB (2003) Ocean currents mediate evolution in island lizards. *Nature* **426**, 552-555.

Campos PF, Kristensen T, Orlando L*, et al.* (in press) Ancient DNA sequences point to a large loss of mitochondrial genetic diversity in the saiga antelope (*Saiga tatarica*) since the Pleistocene. *Molecular Ecology*.

Capello V (2008) Diagnosis and Treatment of Dental Disease in Pet Rodents. *Journal of Exotic Pet Medicine* **17**, 114-123.

Chan YL, Lacey EA, Pearson OP, Hadly EA (2005) Ancient DNA reveals Holocene loss of genetic diversity in a South American rodent. *Biology Letters* **1**, 423-426.

Cooper A, Poinar HN (2000) Ancient DNA: Do It Right or Not at All. *Science*

289, 1139b-.

Dansgaard W, Johnsen SJ, Clausen HB*, et al.* (1993) Evidence for general instability of past climate from a 250-kyr ice-core record. *Nature* **364**, 218-220.

Dávalos LM, Turvey ST (in press) West Indian mammals: the old, the new, and the recently extinct. In: *Historical Biogeography of the Neotropics* (ed. Patterson BD). Chicago University Press, Chicago.

Drummond A, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**, 214.

Felsenstein J (1981) Evolutionary trees from DNA sequences: A maximum likelihood approach. *Journal of Molecular Evolution* **17**, 368-376.

Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**, 783-791.

Felsenstein J (1993) *Phytogeny Inference Package (PHYUP). Version 3.5.* Department of Genetics, University of Washington, Seattle.

Gilbert MTP, Bandelt H-Jr, Hofreiter M, Barnes I (2005a) Assessing ancient DNA studies. *Trends in Ecology & Evolution* **20**, 541-544.

Gilbert MTP, Barnes I, Collins MJ*, et al.* (2005b) Long-term survival of ancient DNA in Egypt: Response to Zink and Nerlich (2003). *American Journal of Physical Anthropology* **128**, 110-114.

Gilbert MTP, Binladen J, Miller W*, et al.* (2007) Recharacterization of ancient DNA miscoding lesions: insights in the era of sequencing-by-synthesis. *Nucleic Acids Research* **35**, 1-10.

Gilbert MTP, Hansen AJ, Willerslev E*, et al.* (2003) Characterization of Genetic Miscoding Lesions Caused by Postmortem Damage. *The American Journal of Human Genetics* **72**, 48-61.

Greenwood AD (2009) Ancient DNA and the Genetic Consequences of Late Pleistocene Extinctions. In: *American Megafaunal Extinctions at the End of the Pleistocene* (ed. Haynes G), pp. 107-123. Springer Netherlands.

Gurnell J, Hare EJ (2008) Rodents; Order Rodentia. In: *Mammals of the British Isles: Handbook, 4th Edition* (eds. Harris S, Yalden DW), pp. 55 -199. The Mammal Society, Southampton, England.

Haas F, Pointer MA, Saino N*, et al.* (2009) An analysis of population genetic differentiation and genotype–phenotype association across the hybrid zone

of carrion and hooded crows using microsatellites and MC1R. *Molecular Ecology* **18**, 294-305.

Hadly EA, Ramakrishnan U, Chan YL*, et al.* (2004) Genetic Response to Climatic Change: Insights from Ancient DNA and Phylochronology. *PLoS Biology* **2**.

Hall BG (2005) *Phylogenetic Trees Made Easy A How-To Manual*, 2nd edn. Sinauer Associates, Inc.

Hartenberger J-L (1998) Description de la radiation des Rodentia (Mammalia) du Paléocène supérieur au Miocène; incidences phylogénétiques. *Comptes Rendus de l'Acadèmie des Sciences Paris, Sciences de la terre et des planètes* **326**, 439-444.

Hasegawa M, Kishino H, Yano T-a (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **22**, 160-174.

Hedges BS (2001) Biogeography of the West Indies: An Overview. In: *Biogeography of the West Indies Patterns and Perspectives* (eds. Woods CA, Sergile FE), pp. 15 - 35. CRC Press, New York.

Hedges SB (1996) Historical Biogeography of West Indian Vertebrates *Annual Review of Ecology and Systematics* **27**, 163-196.

Hedges SB (2006) Paleogeography of the Antilles and Origin of West Indian Terrestrial Vertebrates *Annals of the Missouri Botanical Garden* **93**, 231-244.

Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* **58**, 247-276.

Höss M, Jaruga P, Zastawny TH, Dizdaroglu M, Pääbo S (1996) DNA damage and DNA sequence retrieval from ancient tissues. *Nucleic Acids Research* **24**, 1304-1307.

Huchon D, Catzeflis FM, Douzery EJP (2000) Variance of molecular datings, evolution of rodents and the phylogenetic affinities between Ctenodactylidae and Hystricognathi. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **267**, 393-402.

Irwin D, Kocher T, Wilson A (1991) Evolution of the cytochrome *b* gene of mammals. *Journal of Molecular Evolution* **32**, 128-144.

Iturralde-Vincent MA, MacPhee RDE (1999) Paleogeography of he Caribbean Region: Implications for Cenozoic Biogeography. *Bulletin of the American Museum of Natural History* **238**, 1-95.

Johns GC, Avise JC (1998) A comparative summary of genetic distances in the vertebrates from the mitochondrial cytochrome b gene. *Molecular Biology and Evolution* **15**, 1481-1490.

Jukes JC, Cantor TH (1969) Evolution of protein molecules. In: *Mammalian Protein Metabolism* (ed. H.N. M), pp. 21-132. Academic Press, New York.

Karanth KJ, Delefosse T, Rakotosamimanana B, Parsons TJ, Yoder AD (2005) Ancient DNA from giant extinct lemurs confirms single origin of Malagasy primates. *Proceedings of the National Academy of Sciences* **102**, 14, 5090-5095

Kay EH, Hoekstra HE (2008) Rodents. *Current Biology* **18**, 406-410.

Kelly CD (2005) Understanding mammalian evolution using Bayesian phylogenetic inference. *Mammal Review* **35**, 188-198.

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**, 111-120.

Kimura M (1981) Estimation of evolutionary distances between homologous nucleotide sequences. *Proceedings of the National Academy of Sciences of the United States of America* **78**, 454-458.

Lalueza-Fox C, Castresana J, Sampietro L*, et al.* (2005a) Molecular dating of caprines using ancient DNA sequences of Myotragus balearicus, an extinct endemic Balearic mammal. BioMed Central.

Lalueza-Fox C, Sampietro ML, Caramelli D*, et al.* (2005b) Neandertal Evolutionary Genetics: Mitochondrial DNA Data from the Iberian Peninsula. *Molecular Biology and Evolution* **22**, 1077-1081.

Lanave C, Preparata G, Saccone C, Serio G (1984) A new method for calculating evolutionary substitution rates. *Journal of Molecular Evolution* **20**, 86-93.

Larson G, Dobney K, Albarella U*, et al.* (2005) Worldwide Phylogeography of Wild Boar Reveals Multiple Centers of Pig Domestication. *Science* **307**, 1618-1621.

Larson G, Cucchi T, Fujita M, *et al.* (2007) Phylogeny and ancient DNA of *Sus*

provides insights into neolithic expansion in Island Southeast Asia and Oceania. *Proceedings of the National Academy of Sciences* **104**, 12, 4834-4839.

Larson G, Liu R, Zhao X*, et al.* (2010) Patterns of East Asian pig domestication, migration, and turnover revealed by modern and ancient DNA. *Proceedings of the National Academy of Sciences* **107**, 7686-7691.

Li W-H, Tanimura M, Sharp P (1987) An evaluation of the molecular clock hypothesis using mammalian DNA sequences. *Journal of Molecular Evolution* **25**, 330-342.

Lisiecki LE, Raymo ME (2005) A Pliocene-Pleistocene stack of 57 globally distributed benthic δ^{18}O records. *Paleoceanography* **20**, PA1003.

MacPhee RDE, Iturralde-Vincent MA (2005) The Interpretation of Caribbean Paleogeography: Reply to Hedges. *Insular Vertebrate Evolution*, 175 - 184.

MacPhee RDE, White JL, Woods CA (2000) New Megalonychid Sloths (Phyllophaga, Xenarthra) from the Quaternary of Hispaniola. *American Museum Novitates*, 1-32.

Milankovic M (1969) *Canon of insolation and the ice-age problem (Koniglich Serbische Akademie, Beograd, 1941)* English translation by the Israel Program for Scientific Translations, Jerusalem.

Miller W, Drautz DI, Janecka JE*, et al.* (2009) The mitochondrial genome sequence of the Tasmanian tiger (Thylacinus cynocephalus). *Genome Research* **19**, 213-220.

Millien V, Bovy H (2010) When teeth and bones disagree: body mass estimation of a giant extinct rodent. *Journal of Mammalogy* **91**, 11-18.

Morgan GS, Woods CA (1986) Extinction and the zoogeography of West Indian land mammals. *Biological Journal of the Linnean Society* **28**, 167-203.

Olivieri GL, Sousa V, Chikhi L, Radespiel U (2008) From genetic diversity and structure to conservation: Genetic signature of recent population declines in three mouse lemur species (Microcebus spp.). *Biological Conservation* **141**, 1257-1271.

Pääbo S (1989) Ancient DNA: extraction, characterization, molecular cloning, and enzymatic amplification. *Proceedings of the National Academy of Sciences of the United States of America* **86**, 1939-1943.

Pääbo S, Poinar H, Serre D, *et al.* (2004) Genetic Analyses from Ancient DNA *Annual Review of Genetics* **38**, 645-679.

Pereira F, Carneiro J, van Asch B (2010) A Guide for Mitochondrial DNA Analysis in Non-Human Forensic Investigations. *The Open Forensic Science Journal* **3**, 33-44.

Poinar HN, Höss M, Bada JL, Pääbo S (1996) Amino Acid Racemization and the Preservation of Ancient DNA. *Science* **272**, 864-866.

Posada D, Crandall K (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817-818.

Ramakrishnan U, Hadly EA (2009) Using phylochronology to reveal cryptic population histories: review and synthesis of 29 ancient DNA studies. *Molecular Ecology* **18**, 1310-1330.

Rinderknecht As, Blanco RE (2008) The largest fossil rodent. *Proceedings of the Royal Society B: Biological Sciences* **275**, 923-928.

Ripplinger J, Sullivan J (2008) Does Choice in Model Selection Affect Maximum Likelihood Analysis? *Systematic Biology* **57**, 76-85.

Roca AL, Kahila Bar-Gal G, Eizirik E, *et al.* (2004) Mesozoic origin for West Indian insectivores. *Nature* **429**, 649-651.

Rohland N, Hofreiter M (2007) Comparison and optimization of ancient DNA extraction. *Biotechniques* **42**, 343-352.

Rosen DE (1975) A Vicariance Model of Caribbean Biogeography. *Systematic Zoology* **24**, 431-464.

Rosen DE (1985) Geological Hierarchies and Biogeographic Congruence in the Caribbean. *Annals of the Missouri Botanical Garden* **72**, 636-659.

Shackleton NJ, Fairbanks RG, Chiu T-c, Parrenin F (2004) Absolute calibration of the Greenland time scale: implications for Antarctic time scales and for Δ14C. *Quaternary Science Reviews* **23**, 1513-1522.

Shapiro B, Drummond AJ, Rambaut A, *et al.* (2004) Rise and fall of the Beringian steppe bison. *Science* **306**, 1561-1565.

Sigwart J (2009) Coalescent Theory: An Introduction. *Systematic Biology* **58**, 162-165.

Smith CI, Chamberlain AT, Riley MS, Stringer C, Collins MJ (2003) The thermal history of human fossils and the likelihood of successful DNA amplification. *Journal of Human Evolution* **45**, 203-217.

Stewart JR, Lister AM (2001) Cryptic northern refugia and the origins of thee modern biota. *Trends in Ecology and Evolution* **16**, 608-613.

Stott L, Timmermann A, Thunell R (2007) Southern Hemisphere and Deep-Sea Warming Led Deglacial Atmospheric CO2 Rise and Tropical Warming. *Science* **318**, 435-438.

Svendsen JI, Alexanderson H, Astakhov VI*, et al.* (2004) Late Quaternary ice sheet history of northern Eurasia. *Quaternary Science Reviews* **23**, 1229-1271.

Svensson A, Andersen KK, Bigler M*, et al.* (2006) The Greenland Ice Core Chronology 2005, 15-42 ka. Part 2: comparison to other records. *Quaternary Science Reviews* **25**, 3258-3267.

Swofford DL, Olsen GJ, Waddell PJ, Hills DM (1996) Phylogenetic Inference. In: *Molecular Systematics, Second Edition* (eds. Hillis DM, Moritz C, Mable BK). Sinauer Associates, Massachusetts.

Swofford DL (2000) Phylogenetic analysis using parsimony (* and other methods),. Sinaur Associates, Sunderland, Massachusetts.

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10**, 512-526.

Thomas W, Pääbo S, Villablanca F, Wilson A (1990) Spatial and temporal continuity of kangaroo rat populations shown by sequencing mitochondrial DNA from museum specimens. *Journal of Molecular Evolution* **31**, 101-112.

Valdiosera CE, García-Garitagoitia JL, Garcia N*, et al.* (2008) Surprising migration and population size dynamics in ancient Iberian brown bears (Ursus arctos). *Proceedings of the National Academy of Sciences* **105**, 5123-5128.

Vaughan TA, Ryan JM, Czaplewski NJ (2010) Mammalogy, Fifth Edition, pp. 198-239. Jones and Bartlett Learning, Sudbury.

Waelbroeck C, Labeyrie L, Michel E*, et al.* (2002) Sea-level and deep water temperature changes derived from benthic foraminifera isotopic records. *Quaternary Science Reviews* **21**, 295-305.

Willerslev E, Cooper A (2005) Ancient DNA. *Proceedings of the Royal Society B: Biological Sciences* **272**, 3-16.

Williams EE (1989) Old problems and new opportunities in West Indian biogeography. In: *Biogeography of the West Indies: past, present, and future* (ed. Woods CA), pp. 1-46. Sandhill Crane Press, Gainesville.

Willis KJ (1996) Where did all the flowers go? THe fate of temperate European flora during glacial periods. *Endeavour* **20**, 110-114.

Wolff EW, Chappellaz J, Blunier T, Rasmussen SO, Svensson A (2010) Millennial-scale variability during the last glacial: The ice core record. *Quaternary Science Reviews* **29**, 2828-2838.

Yang DY, Eng B, Waye JS, Dudar JC, Saunders SR (1998) Improved DNA extraction from ancient bones using silica-based spin columns. *American Journal of Physical Anthropology* **105**, 539-543.

Yang Z, Rannala B (1997) Bayesian phylogenetic inference using DNA sequences: a Markov Chain Monte Carlo Method. *Molecular Biology and Evolution* **14**, 717-724.

Yoder A, Yang Z (2000) Estimation of Primate Speciation Dates Using Local Molecular Clocks. *Molecular Biology and Evolution* **17**, 1081-1090.

Zharkikh A (1994) Estimation of evolutionary distances between nucleotide sequences. *Journal of Molecular Evolution* **39**, 315-329.

Zuckerkandl E, Pauling L (1965) Evolutionary divergence and convergence in proteins. In: *Evolving genes and proteins* (eds. Bryson V, Vogel H), pp. 97-166. Academic Press, New York.

Chapter 2: Arctic collared lemming in northern Europe

2.1 Abstract

The extinction of many large mammal fauna at the end of the Pleistocene has inspired scientific research since the beginning of the nineteenth century, resulting in multiple theories on causality. In a novel approach to explore Late Pleistocene extinction events I employ ancient DNA techniques to examine an Arctic adapted species, the arctic collared lemming (*Dicrostonyx torquatus*), from a single archaeological site in Europe, during the end of the Late Pleistocene. Phylogenetic analysis using mitochondrial DNA (cytochrome *b*) reveals three genetically distinct lineages of arctic collared lemming, separated into three discrete time periods. This structure is indicative of genetic replacement, through population extinction followed by population recolonisation. I further utilise the phylogenetic structure and timing of the population turnover events observed, to contrast with the most thoroughly dated Late Pleistocene extinction in Europe, that of the woolly mammoth (*Mammuthus primigenius*). The results indicate that timing of arctic collared lemming extinction and replacement events correspond simultaneously with localised disappearance of the woolly mammoth. A finding that favours a non-selective explanation for the turnover events and absences observed and suggests the influential role of climatic and environmental change.

2.2 Introduction

The beginning of the nineteenth century witnessed a significant increase in knowledge of extinct animal faunas. Excavation and analysis of fossil material resulted in the realisation that relatively recently, in reality between 50,000 and 10,000 years ago, previously unknown large mammal fauna roamed the earth. Iconic images of woolly mammoths (*Mammuthus primigenius*) captured the imagination of the general public, and promoted the importance of the new discipline of palaeontology (Grayson, 1984). Within the scientific community, a debate began that continues to this day; what caused an estimated 97 genera to become globally extinct, a total that increases to 121 genera when continent wide

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extinctions are included (Barnosky *et al.*, 2004). That so many large bodied mammal lineages were extinguished within what can be considered a relatively short space of time has become known as 'the megafaunal extinction event'. Theories on causality are both multiple and extensively reviewed (Barnosky *et al.*, 2004; Koch, Barnosky, 2006).

Two main hypotheses, however, focus on anthropogenic or climatic changes either exclusively or in combination. Many scientists, upon the recognition that humans and megafauna once co-existed, concluded that hunting by early human was the most likely explanation for the extermination of prehistoric large mammal fauna (Grayson, 2008). Martin (1966) was an early proponent of the overkill hypothesis, which, in its most basic form infers that the rate at which a species is hunted exceeds the rate at which the species is capable of reproducing. 'Prehistoric blitzkrieg' was developed over a series of papers (Martin, 1973; Martin, 1984) proposing that early human settlers (particularly in the Americas) selectively, heavily and rapidly hunted the abundant large bodied prey, which was easy to kill due to prey naivety (lack of experience to the threat of hunting). This culminated in the rapid growth of human populations and rapid extinction of the large mammal fauna.

More recently, Lyons (2004) and Barnosky *et al.* (2004) stress the concurrence of Pleistocene extinction events with continental colonisation by aboriginal humans, and the overkill hypothesis has been shown viable through simulation modelling (Alroy, 2001). Others, however, are diametrically opposed to the overkill model (Wroe *et al.*, 2004) and identify the rarity of megafaunal kill sites (Grayson, Meltzer, 2003), general lack of evidence that proboscidians in particular were regularly utilised as a food source (Gaudzinski *et al.*, 2005) and the non-synchronous timing of extinctions events (Grayson, 2008) as points against the overkill hypothesis.

Hypotheses relating to anthropogenic influence are not limited to hunting; the sitzkreig model (Diamond, 1989) advocates a slower attrition, implicating additional characteristics of human colonisation e.g. fire, habitat fragmentation, exotic species and disease introductions. While the hyperdisease model

(MacPhee, Marx, 1997) specifically advocates the role of one or more highly virulent diseases introduced by early humans to susceptible 'naïve' populations of native mammals. Lyons *et al.* (2004), however, analysed avian and West Nile virus data to empirically test a disease that conforms to the criteria of a hyperdisease, but found no evidence that it would cause a size-biased extinction event.

The principle alternative category of hypothesised causality attributes megafaunal extinctions to extensive environmental and climatic changes, particularly those associated with the transition from the last glacial cycle into the warmer current epoch (the Holocene) (ca. 11.5 Kyr BP). These changes would have had a profound ecological effect on the environment, altering vegetation and habitat. This resulted in the loss or fragmentation of suitable habitat for megafauna species, reducing or eliminating resources, rendering populations unviable and ultimately leading to extinction (Barnosky, 1986; Ficcarelli *et al.*, 2003; Pushkina, Raia, 2008; Stuart *et al.*, 2004).

More recently a novel environmental hypothesis has gained favour, that of an extraterrestrial impact (Firestone, 2009; Firestone *et al.*, 2007; Kennett *et al.*, 2009). Advocates proposed that a large comet fragmented and exploded over the Laurentide Ice Sheet ca. 12.9 Kyr BP, the impact of which contributed to abrupt environmental changes such as the onset of the Younger Dryas cooling (ca. 12.8 – 11.5 Kyr BP), and was the underlying cause of the extirpation of the megafauna in North America. However, the carbon spherules and 'glass-like carbon', regarded by Firestone and Kennett as exclusive by-products of the wildfires ignited by the impact, have recently been identified as fungal sclerotia, common in both modern and ancient deposits (Scott *et al.*, 2010). Further, the identification of crucial impact markers, nanodiamonds, reported in Younger Dryas sediments have been called into question. Daulton *et al.* (2010) used additional quantification techniques and found no evidence of lonsdaleite or any other nanodiamonds from the same locality as previously reported. The presence of nanodiamonds, and in particular the rare polytype of diamond, lonsdaleite, were pivotal to the impact hypothesis as they are associated with shock pressures that occur upon the impaction of a meteorite.

The timing of extinction events in North America persists as a controversial issue, were extinctions synchronous (Faith, Surovell, 2009) or staggered (Grayson, 2007). In Eurasia, however, it is generally accepted that events occurred in a piecemeal fashion, with individual taxa disappearing at different times across geographical locations. The giant deer (*Megaloceros giganteus*) was once widespread across western and central Europe, it disappeared from the region ca. 24 Kyr BP ago (Stuart *et al.*, 2004; all dates herein are reported as calibrated dates, see *Appendix A* for any uncalibrated dates referenced in the literature) but returned to the northwest of Europe ca. 13.7 Kyr BP. It continued to persist in central Europe until its extinction ca. 12 Kyr BP. A population in the Urals and western Siberia however survived well into the Holocene, with the last known dates occurring ca. 7.9 Kyr BP (Stuart *et al.*, 2004). In contrast, the straight-tusked elephant (*Palaeoloxodon antiquus*), found throughout western Europe during interglacials survived at the latest until ca. 39-38 Kyr BP (Stuart, 2005) in Iberia, but did not persist into the Last Glacial Maximum (LGM) ca. 27–23Kyr BP. A range of last occurrence dates from south western France (Grayson, 2007) further highlight the non-synchronous nature of the Eurasian extinctions, the woolly rhinoceros (*Coelodonta antiquitatis*) for example survived until ca. 17 Kyr BP, while the muskox (*Ovibos moschatus*) had disappeared by 23 Kyr BP (Grayson, 2007).

The fate of the woolly mammoth has spawned a particularly vibrant field of research, likely due to an abundance of bone collections, with frequent new finds. The size of mammoth bones made them more amenable to pre Accelerator Mass Spectrometry (AMS) radiocarbon dating techniques and because they are extinct, there was no risk of a costly error in the accidental dating a modern bone. There exists therefore a wealth of scientific literature, including both radiocarbon dates (Guthrie, 2004; Stuart *et al.*, 2004; Ugan, Byers, 2008) and ancient DNA data at the species, taxonomy and population scales (Barnes *et al.*, 2007; Haile *et al.*, 2009; Krause *et al.*, 2006).

With reference to the Eurasian extinction event, the current body of evidence suggests that mammoth were widespread across northern Eurasia and most of Europe throughout the last cold stage until around ca. 21 Kyr BP (Stuart, 2005).

At this time they disappeared entirely from Western Europe, but returned 2000 years later to occupy an almost identical range, with the exception of Southern Europe, where they failed to recolonise. Persistence in the rest of Europe continued until ca. 13.7 Kyr BP (Stuart, 2005), after which they are considered extinct in central and Western Europe. This final extinction correlates with a loss of suitable habitat, as open biomes disappear with the beginning of the warmer Bølling/Allerød (Lister, Stuart, 2008; Stuart, 2005; Stuart *et al.*, 2004). Material dated from Puurmani, Estonia (Lõugas *et al.*, 2002) indicate an additional re-expansion restricted to northeastern Europe ca. 11.5 Kyr BP. The timing of this coincides with the return of a steppe environment to this location at the onset of the Younger Dryas.

Survival into the Holocene seems to have been restricted to northern Siberia, with the last known populations occurring on islands; St Paul (or Pribilov) Island, where the youngest mammoth date to ca. 6.6 Kyr BP (Veltre *et al.*, 2008) and Wrangel Island, dated to ca. 4 Kyr BP (Vartanyan *et al.*, 2008). That the very last mammoths survived on islands in the far North has led to speculation that these provided a safe haven from human predation (Veltre *et al.*, 2008). Alternatively, it may simply reflect that last stand of a species under intense environmental pressure, affected by range contraction, population fragmentation and reduced resources.

Why this particular glacial cycle should result in extinction, when similar conditions had previously occurred, has given rise to combined explanations incorporating both environmental pressure and human predation. Stuart (2005) suggests the possibility of humans impeding 'normal' migration response, while Kuzmin (2009) finds the evidence to date too inconclusive to speculate. In general it appears highly problematic to definitively preclude or separate factors involved in the final mammoth extinction.

The mammoth has proved an impressive model for examining the complexities of the Eurasian megafaunal extinction. As a model to examine the debate between human hunting and climatic change it falls short of providing a clear answer. It therefore seemed appropriate to examine the fate of a different species, one that

was unlikely to have been hunted, in order to assess the effects of climate change in isolation. The arctic collared lemming (*Dicrostonyx torquatus*) fulfils the criteria, because as a small rodent it was unlikely to have been affected by human activities, including hunting, it has well-characterised habitat requirements and is clearly recorded in the fossil record, from the mid to late Pleistocene and into the Holocene throughout Central Europe (Maul, Markova, 2007; Stewart, 2003). Its current absence from Europe eliminates the risk of contamination from modern material and its unique tooth morphology allows for clear identification from archaeological deposits.

Arctic collared lemmings are currently restricted to the Palaearctic tundra, from the White Sea in West Russia, to the Chukotka Peninsula and Northeast Siberia and Kamchatka including Novaya Zemlya and the New Siberian islands (Corbet, 1978). An ecologically significant genus, they form the nutritional dietary basis for the majority of Arctic tundra birds of prey as well as carnivorous mammals. As a tundra specialist the arctic collared lemmings' habitat range expanded south during glacial advances, extending suitable habitat into central Europe, contracting north to their current location, postglacial retreat.

Previous phylogenetic research concerning lemming demography has reported low nucleotide and haplotype diversity in present day populations with geographic constraints on individual haplotypes (Fedorov *et al.*, 1999; Prost *et al.*, 2010). This signature is often associated with a population bottleneck, where a declining population losses genetic diversity through reduced effective population size and genetic drift. Habitat contraction has been postulated as a potential cause for prior population decline in the arctic collared lemming (Fedorov, 1999) with post population expansion occurring from a refugial source, potentially from a region east of the Kolyma River (Fedorov, Goropashnaya, 1999). Further evidence of a population bottleneck has recently been confirmed with the utilization of ancient DNA, where the timing of population decline both in effective population size and genetic diversity correlates with abrupt climate change during the Greenland Interstadial 1 (Bølling/Allerød) global warming event approximately 14.7 Kyr BP (Prost *et al.*, 2010).

The inclusion of ancient DNA has proven an effective tool in understanding past events through the identification of population level differences in structure through time and has been widely employed to discern population responses to climatic change (Barnes *et al.*, 2002; Barnes *et al.*, 2007; Shapiro *et al.*, 2004). My research focuses essentially on a single site, Trou Al'Wesse, a prehistoric cave located at the northern edge of the Belgian Ardennes in the Hoyoux river valley (Modave, Liège Province) (*Figure 2.1*).

Figure 2.1 *Location of Trou Al'Wesse cave site*
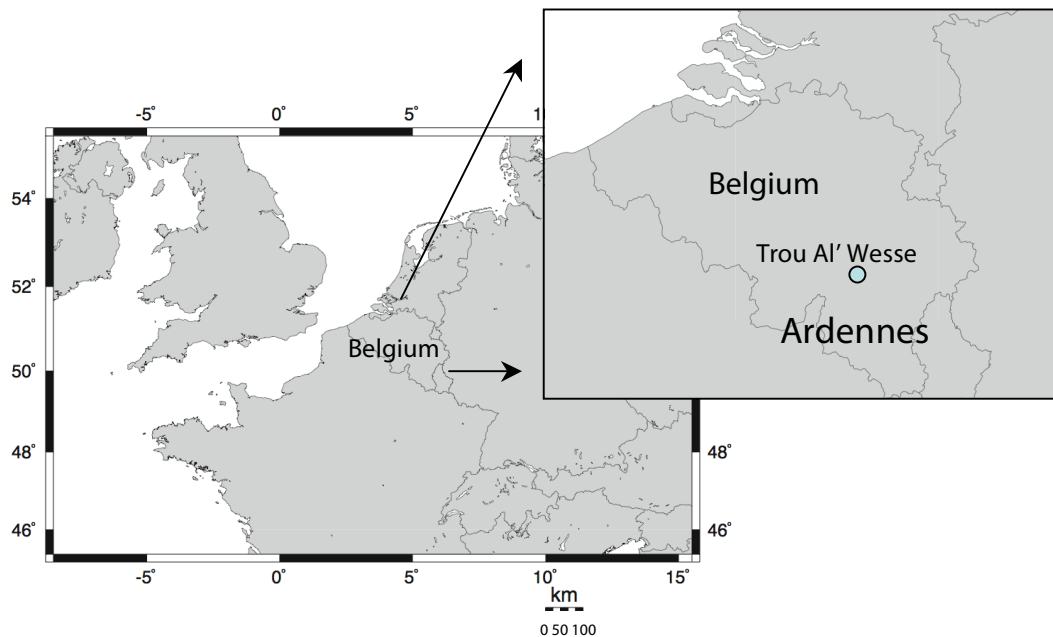
The blue circle denotes the location of the Trou Al' Wesse site in Belgium

This area provides a unique perspective as it approaches the most southerly limits of arctic collared lemming habitat range expansion during glacial intervals. Thus, it provides data on an ecologically important species in a region where sensitivity to environmental change has been heightened by relative proximity to the range margins. This study further benefits from a phylochronological approach – using phylogenetic methods to examine a population through space and time - sampling a single location through time, including the period before, during and after the LGM. This therefore encompasses periods of significant environmental change.

The continued advancement of knowledge in a key prey species, examining the long-term history of environmental change responses, is of wider interest for a suite of other cold-stage taxa and can be utilized in predictions and possible management strategies concerning future climatic change. In addition it holds potential to add comment to a 200-year-old debate, the cause of the megafaunal extinctions. The study of ancient DNA from a small mammal species at the same time, geographic region and under the same environmental stresses as that of the large cold adapted fauna permits comparative analysis. It also affords an opportunity to remove the effects of human hunting and focus on climatic events in isolation during a period of dramatic environmental change and extinctions.

2.3 Materials and methods

2.3.1 Samples

A total of 60 arctic collared lemming mandibles were obtained from Trou Al'Wesse (TAW; 50.421, 5.294), a prehistoric archeological cave site in the Belgian Ardennes, located 50 meters from the Hoyoux River. Excavation of the cave entrance began in 1864 by Édouard Dupont, resulting in a single trench, this was followed by a tunnel in 1885-87 dug by Julien Fraipont, Max Lohest and Ivan Braconnier. Modern excavations of a long trench and test pits were carried out between 1988 and 2001, under the direction of M. Otte and F. Collin. The current phase of excavations, directed by R. Miller, began in 2003, and has concentrated on the expansion of the terrace zone (Miller *et al.*, 2009) (*Figure 2.2*).

TAW continues to be an active excavation site, the current stratigraphic sequence spanning approximately 60,000 to 5,000 years BP is divided into geologically well-defined strata, containing Mousterian, Aurignacian, Mesolithic and Neolithic occupations with intervening periods rich in faunal material (*Figure 2.3*). In order to examine faunal response to events surrounding the LGM, and to remain within accepted limits for radiocarbon dating, arctic collared lemming samples were obtained from pre LGM layers (16 and 14) up to and including transition into the warmer climate of the Holocene (layer 4).

Figure 2.2 Site plan of TAW

Modified from Miller et al. (2009)
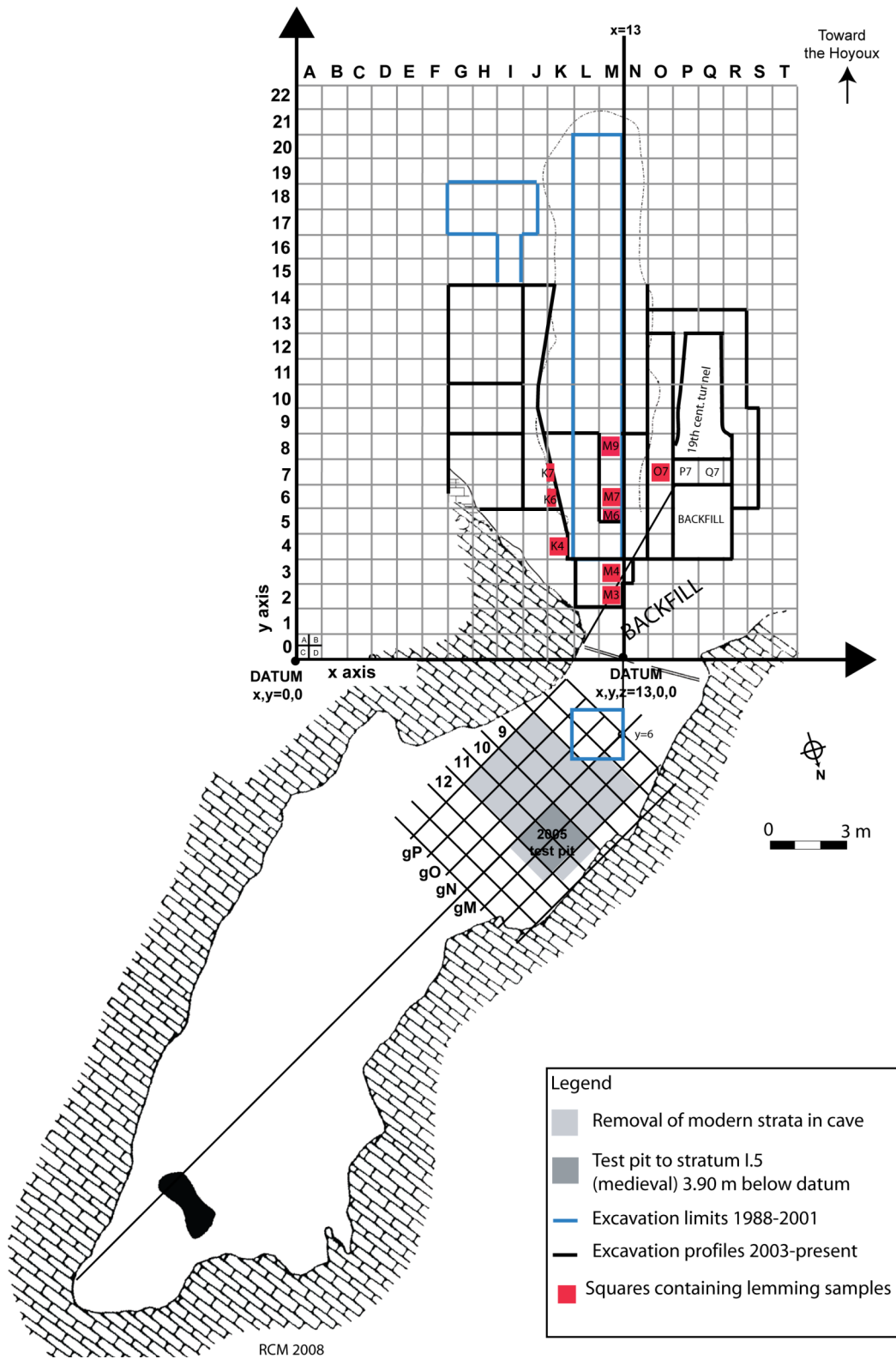
Figure 2.3 *Section profile of TAW*

Layers

5045BP
5950BP
6650BP

Mesolithic

30750BP

36500BP

Aurignacian

Mousterian

41100BP

meters
below datum

A   B   C   D

▱ Dolomite
● Pebble
○ Displaced stone
▨ Sand
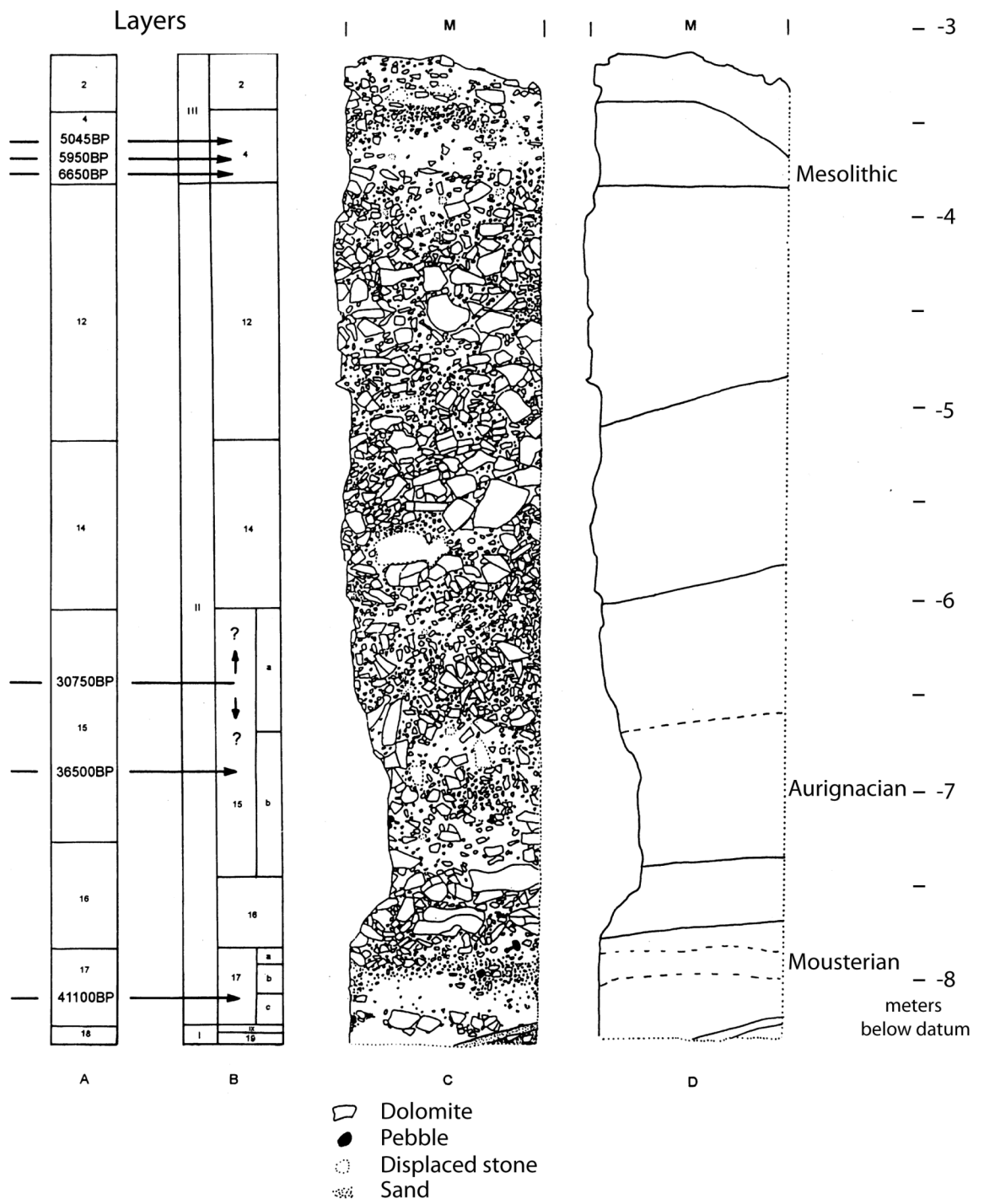
Modified from Pirson (1999)

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The site plan of ongoing excavation (*Figure 2.2*) illustrates the site configuration and indicates the relative location of samples investigated in this study, according to their square of origin. Meter long grid squares define the lateral plane of the site, each sub-squared into 50cm grids. The vertical plane is delineated by the series of geological strata (*Figure 2.3*); each stratum divided into a series of 5-10cm depth spits. Samples utilized in this study can therefore be accurately described with regards to their location of origin within the stratigraphic layers (*Table 2.1* for full details *Appendix B* also *Appendices C - F* for site profile locations of samples).

Five additional arctic collared lemming mandibles were obtained from Bridged Pot cave (51.234, -2.680) an archeological site located in Ebbor Gorge, Somerset, England. Samples were sourced from an established stratigraphic layer associated with the Younger Dryas horizon (ca. 12.8 – 11.5 Kyr BP) providing additional data from a younger site with proximity to TAW, 570 Km, heading 282 degrees.

All materials were sieved on site during excavations (2005 – 2007), sealed in bags and maintained at a constant temperature to limit contamination and DNA degradation. In the laboratory, samples were identified to species level through morphological mandibular characters in the M_1 tooth, species specific to the arctic collared lemming (Nadachowski, 1982).

Table 2.1 *Stratigraphic locations of arctic collared lemming samples*

Country	Site	Layer	Square	Spit	Number of samples
Belgium	TAW	4	K4a	5	1
Belgium	TAW	4	K4b	5	1
Belgium	TAW	12	M3	1	8
Belgium	TAW	12	M3	2	3
Belgium	TAW	12	M3	9	6
Belgium	TAW	12	M4	5	12
Belgium	TAW	12	M4	6	15
Belgium	TAW	14	O7	16	3
Belgium	TAW	14	K6	9	2
Belgium	TAW	14	K7	9	1
Belgium	TAW	15B	M9	8	3
Belgium	TAW	15B	M9	10	1
Belgium	TAW	16	M6	1	1
Belgium	TAW	16	M7	16	2
Belgium	TAW	16	M7	19	1
UK	Bridged Pot cave				5
				Total	65

2.3.2 DNA extraction

All DNA extractions were conducted in a dedicated ancient DNA laboratory, physically separated from the post- PCR laboratory. Mandibles were ground into a fine powder and extracted using silica spin columns based on Yang *et al*. (1998) with the inclusion of 1M urea in the extraction buffer. Mitochondrial DNA (mtDNA) was amplified using overlapping fragments spanning 780 base pairs of the cytochrome b region. Nine primer pairs were designed specifically for this study (*Appendix G*), each pair amplifying short (130 – 180 base pair) overlapping fragments. PCR reactions were performed using a final concentration of 1 x PCR buffer, $0.2\mu M$ of each primer, $250\mu M$ dNTPs, $2mM$ $MgSO_4$, $1mg/ml$ BSA, 1 Unit Platinum Taq DNA polymerase high fidelity, purified water, and $2\mu l$ of DNA extract in a $25\mu l$ mix. PCR conditions were 5 min at 95°C, followed by 55 cycles of 1 min at 92°C, 1 min at 50°C or 51°C (dependent on primer pair specifications), 1 min at 68°C, and with a final extension of 5 min at 68°C.

Amplicons were purified using Exonuclease I and Shrimp Alkaline Phosphatase. Sequencing reactions were performed by Macrogen (Korea) using a high

throughput genetic analysis sequencer, ABI3730XL. Sequencing chromatograms were assembled and analysed using Sequencher 4.0 analysis software (Gene Codes Corporation). Standard ancient DNA protocols (Gilbert *et al.* 2005) were followed throughout these extraction procedures to prevent contamination, with repeated PCR amplification and sequencing of fragments to ensure DNA authenticity and the absence of miscoding lesions.

2.3.3 Phylogenetic analyses

Ancient DNA sequences were aligned by eye in Se-AL 2.0 with all modern arctic collared lemming sequences available on Genbank plus a single northern collared lemming (*Dicrostonyx groenlandicus*) sequence (*Appendix H*).

Phylogenetic relationships were estimated using Maximum Likelihood (ML) and Bayesian analysis. The DNA substitution model selected with ModelTest3.7 (Posada, Crandall, 1998) under Akaike Information Criterion (AIC) was Hasegawa-Kishino-Yano (HKY) (Hasegawa *et al.*, 1985) with proportion of invariable sites (I) set to 0.556 and gamma distribution (G) shape parameter 0.8868.

Maximum Likelihood trees were generated in PAUP* (Swofford, 2000) using a full heuristic search, based on branch swapping with tree-bisection-reconnection (TBR). Bayesian trees were constructed using MrBayes 3.1 (Ronquist, Huelsenbeck, 2003) implementing nucleotide substitution model HKY, four chains (three heated one cold) were run for one million generations with a burn-in period of 500 trees. Nodal support was determined using ML bootstrap analysis with 500 replicates in PAUP* and by approximate posterior probabilities performed in MrBayes. The northern collared lemming was utilized as the outgroup for all analyses.

To investigate potential source populations, additional sequence data from Pymva-Shor (PS) a site in the northern Pre-Urals were taken from Prost *et al.* (2010). The sequence data from PS were from the cytochrome b region but of shorter length than those generated in this study, thus only a subsection of TAW data were employed in these analyses, 280 base pairs. Methods were identical,

except the DNA substitution model selected under AIC in Modeltest was HKY, with no invariable sites and gamma distribution shape parameter 0.2072.

For the TAW data, sequence diversity parameters including number of haplotypes, transitions and transversions, and nucleotide and haplotype diversity were calculated in Arlequin Ver. 3.11 (Excoffier *et al.*, 2005).

2.3.4 Radiocarbon Dating

The quantity of bone powder utilized in the DNA extraction process was experimentally reduced over successive trials, from a standard 50mg to 11mg. This provided additional bone material availability for radiocarbon dating, permitting direct radiocarbon dating of samples used in these analyses.

After successful sequencing, the remaining bone materials were assessed for suitability for radiocarbon dating on the basis of weight and likely collagen preservation. Following discussion with Dr Tom Higham, (deputy director) at the Oxford Radiocarbon Accelerator Unit (ORAU), 13 arctic collared lemming samples were identified for radiocarbon dating. Accelerator Mass Spectrometry (AMS) dating was carried out at the ORAU, where samples were chemically pre-treated and ultra-filtered to remove potential contaminants before radiocarbon measurements were taken on the AMS system.

2.3.5 Dating analyses

Directly dated samples were provided with dates in uncalibrated radiocarbon years BP (Before Present AD 1950) format. Calibrated calendar ages were generated using Oxcal v4.1 (Ramsey, 2009) with IntCal09 calibration curve (Reimer *et al.*, 2009).

In order to compare the TAW arctic collared lemmings with a larger extinct mammal, directly dated mammoth radiocarbon dates were sourced from the ORAU database. A literature search for recent (post 1985) publications and reviews containing mammoth dates were also undertaken, to include the bulk of contemporary and most reliably dated material. To assure spatiotemporal relevance, criteria for selection were both geographical (all samples west of

Russia) and temporal (all dates younger than 30 Kyr uncalibrated BP; *Figure 2.4 and Table 2.2*). To identify patterns directly related to the TAW locale, mammoth dates were further partitioned according to distance from TAW, with a boundary set at an arbitrary 1000 km radius from the site. Distance between TAW and mammoth sites were calculated using the 'haversine' formulae, an equation to calculate great-circle distances between longitude and latitude points on a sphere (Veness, 2002-2010). Calendar dates and calibration plots for the mammoth were generated in OxCal.

Figure 2.4 *Mammoth sampling from Europe and the 1000 Km inclusion zone*
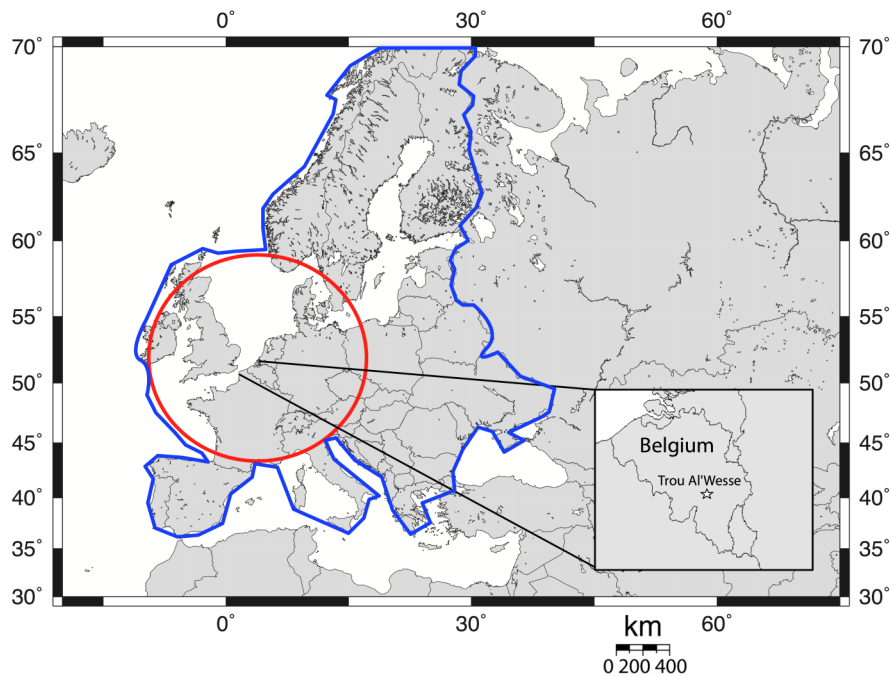
The blue line denotes the area targeted to search for mammoth data, the red circle denotes the 1000 Km partitioning zone for mammoth considered local to TAW.

Table 2.2 *Mammoth radiocarbon dates sourced from the literature*

Country	Site	Lab reference	Date	+/-	Longitude	Latitude	Distance (Km) from TAW	Reference
Estonia	Puurmani	Hela-423/425	10150	100	26.28	58.57	1619	Lõugas *et al.* (2002)
Germany	Gonnersdorf	OxA-2069	11830	120	7.46667	50.4167	150.4	Stevens *et al.* (2009)
UK	Condover	OxA-20129	12230	50	-2.75	52.65	608.6	Scource *et al.* (2009)
France	Buisson Campin a Verberie	OxA-12018	12235	75	2.73	49.31	221.4	Lister (2009)
UK	Condover	OxA-1316	12300	180	-2.75	52.65	608.6	Hedges *et al.* (1989)
France	Etiolles, Paris Basin	OxA-12019	12315	55	2.47	48.63	285	Barnes *et al.* (2007)
UK	Robin Hood's Cave	OxA-1462	12320	120	-1.19	53.27	546.2	Stuart *et al.* (2002)
UK	Condover	OxA-1456	12330	120	-2.75	52.65	608.6	Hedges *et al.* (1989)
UK	Condover	OxA-19903	12375	50	-2.75	52.65	608.6	Scource *et al.* (2009)
UK	Pin Hole Cave	OxA-1204	12460	160	-1.1957	53.264	546.2	Hedges *et al.* (1988)
UK	Gough's Cave	OxA-17846	12470	55	-2.76559	51.2817	573.5	Jacobi & Higham (2009)
Czech Republic	Pekarna	GrN-14828	12670	80	16.75	49.24	831.1	Lister (2009)
Ukraine	Dobranichevka	OxA-700	12700	200	34.98	48.59	2139	Gowlett *et al.* (1987)
France	Marolles-sur-Seine	OxA-12020	12800	65	3.03	48.37	280.8	Lister (2009)
Ukraine	Mezhirich	OxA-709	12900	200	34.48	50.69	2049	Gowlett *et al.* (1987)
Sweden	Lockarp	Poz 3941	13310	60	13.03	55.51	766.3	Ukkonen *et al.* (2007)
France	la Colombiere Rockshelter	Ly 433	13390	300	5.37	46.08	482.8	Stuart *et al.* (2002)
Switzerland	Praz Rodet	OxA-12982	13705	55	6.93	46.95	404.2	Lister (2009)
Ukraine	Obukhov Town	OxA-11974	13945	50	30.64	50.1	1793	Barnes *et al.* (2007)
Switzerland	Kesslerloch Cave	OxA-10237	13980	110	8.79	47.63	401.5	Stuart *et al.* (2002)
Germany	Oelknitz	oxA-10240	14100	100	11.61	50.84	447.8	Stuart *et al.* (2002)
Germany	Gonnersdorf	OxA-10239	14380	100	7.41667	50.45	150.4	Stevens *et al.* (2009)
Czech Republic	Pekarna Cave	Oxa-11353	14400	90	16.75	49.24	831.1	Lister (pers. comm)
Ukraine	Mezhirich	OxA-712	14400	250	34.48	50.69	2049	Gowlett *et al.* (1987)
Germany	Kniegrotte	OxA-4851	14470	140	11.55	50.6667	442.8	Hedges *et al.* (1998)
Germany	Gonnersdorf	OxA-10199	14570	90	7.41667	50.45	150.4	Stevens *et al.* (2009)
Ukraine	Chulatovo I	OxA-715	14700	250	33.25	52	1943	Gowlett *et al.* (1987)
Switzerland	Kesslerloch Cave	Oxa-10298	15020	180	8.79	47.63	401.5	Lister (pers. comm)
Belarus	Berdyzh	OxA-716	15100	250	30.95	52.84	1781	Gowlett *et al.* (1987)
Ukraine	Mezin	OxA-719	15100	200	33.08	51.81	1934	Gowlett *et al.* (1987)
Finland	Herttoniemi	Hel-1074	15500	200	25.03	60.2	1644	Stuart *et al.* (2002)
Finland	Herttoniemi	Hela-321	15910	155	25.03	60.2	1644	Ukkonen *et al.* (1999)
Moldova	Cosauti 1	Oxa-12000	17720	80	28.27	48.21	1677	Lister (pers. comm)
Poland	Oblazowa Cave	OxA-3694	18160	260	20.1667	49.4167	1069	Hedges *et al.* (1996)
Ukraine	Radomyshl'	OxA-697	19000	300	29.24	50.5	1688	Gowlett *et al.* (1987)
Spain	Cueto de la mina	OxA-10122	19700	500	-4.84	43.42	1093	Stuart *et al.* (2002)
Ukraine	Novgorod-Severskii	OxA-698	19800	350	33.27	52	1945	Gowlett *et al.* (1987)
Poland	Krakow	OxA-635	20200	350	19.94	50.06	1041	Gowlett *et al.* (1986)
Ireland	Castlepook Cave	OxA-4233	20360	220	-8.58333	52.2167	983.2	Hedges *et al.* (1997)
Germany	Wildscheuer Cave	OxA-7498	20480	360	8.137	50.421	201.4	Hedges *et al.* (1998)
France	Villefranche-s-Saone	OxA-4093	21330	240	4.72	45.98	495.7	Hedges *et al.* (1997)
Finland	Nilsiä, Syväri	Hela-281	22420	315	28.11	63.22	1968	Ukkonen *et al.* (1999)
Finland	Töölö	Hela-282	23340	350	24.93	60.16	1637	Ukkonen *et al.* (1999)
Ukraine	Pogon Palaeolithic Site	OxA-11746	23560	140	33.28	52.21	1943	Barnes *et al.* (2007)
Denmark	Kamstrup, Copenhagen	OxA-10662	23810	260	12.08	55.61	733.4	Lister (pers. comm)
Germany	Gollwitz, Poel	KIA-35747	23900	130	11.48	54.02	580.7	Sommer & Benecke (2009)
Belgium	Third Cave, Goyet	OxA-11292	23940	180	4.98	50.45	22.47	Barnes *et al.* (2007)
Finland	Lohtaja	Hela-295	24450	383	23.48	64.03	1853	Ukkonen *et al.* (1999)
Sweden	Sollefteå, Västernorrland	LuS 6328	24750	200	17.27	63.17	1588	Ukkonen *et al.* (2007)
Germany	Vogelherd Cave	OxA-10196	25780	250	10.2	48.56	410.2	Lister (pers. comm)
Sweden	Pilgrimstad, Jämtland	LuS 6330	25900	200	15.05	62.96	1512	Ukkonen *et al.* (2007)
Sweden	Djurslöv, Mölleberga	LuS 6336	26150	200	13.18	55.61	780.8	Ukkonen *et al.* (2007)
UK	Pin Hole Cave	OxA-1205	26700	550	-1.1957	53.264	546.2	Hedges *et al.* (1988)
Ireland	Shandon Cave	OxA-3607	27150	350	-7.5	52.1	908.3	Hedges *et al.* (1997)
Germany	Zarrenthin	KIA-35744	27340	160	10.92	53.55	518.8	Sommer & Benecke (2009)
Italy	Asolo	OxA-12213	27800	300	11.91	45.79	711.2	Lister (pers. comm)
Denmark	Trappeskov 2, Svendborg	OxA-10663	27810	270	10.61	55.06	627.4	Lister (pers. comm)
Germany	Geissenklosterle Cave	OxA-5229	27950	550	9.76667	48.4	393.9	Conrad & Bolus (2008)
Germany	Gohren	KIA-35743	28180	180	13.74	54.34	719.3	Sommer & Benecke (2009)
Germany	Sirgenstein	KIA 13081	28400	200	9.757	48.387	394.2	Conrad & Bolus (2003)
Germany	Geissenklosterle Cave	OxA-5228	28500	550	9.76667	48.4	393.9	Conrad & Bolus (2008)
Finland	Haapajärvi	Hela-294	28740	670	25.29	63.64	1887	Ukkonen *et al.* (1999)
Estonia	Valga	OxA-11607	28780	160	26.05	57.78	1572	Barnes *et al.* (2007)
Sweden	Lerdal, Rättvik, Dalarna	LuS 6331	29450	300	15.11	60.87	1312	Ukkonen *et al.* (2007)
Sweden	Västansjö, Sättna	LuS 6329	29500	250	16.99	62.5	1518	Ukkonen *et al.* (2007)
Germany	Geissenklosterle Cave	KIA 8960	29800	240	9.77	48.4	394.1	Conrad & Bolus (2003)

Samples outside the 1000Km radius of TAW are highlighted blue

2.4 Results

2.4.1 DNA sequence data

From the 65 arctic collared lemmings sampled, only 4 samples failed to yield mtDNA, an additional 4 samples yielded DNA but of a degraded nature, producing insufficient amplification of the region of interest; these 8 samples were therefore omitted from the analyses (*Appendix B*). The remaining 57 samples yielded the entire 780 base pair region targeted for this study. It is interesting to note that all 4 of the failed samples, those that completely failed to amplify DNA, originated in and were the only materials available to sample from layer 15. It has since been postulated (J. Stewart pers. comm.), outside the results of this study, that layer 15 may be fluvial and thus contain older deposited materials. That the samples were of a greater age and thus deterioration likely explains the inability to amplify mtDNA in light of the high rate of success (93%) in the remaining samples.

2.4.2. Phylogenetic analyses

Maximum Likelihood and Bayesian analyses generated highly congruent phylogenetic trees, thus only the Bayesian trees are shown, with both the Bayesian approximated posterior probabilities and ML bootstrap values indicated (*Figure 2.5*). Three well-supported clades are apparent from the data, each pertaining to a time frame in approximate concurrence with the stratigraphy of the site. The topmost layers from TAW (layer 12, spits 1-6) grouping with modern material (coloured black) and those from the Younger Dryas (Bridged Pot cave, coloured peach) in clade 1, the second clade consists of samples from the middle layers of TAW (layer 12, spits 5-9), while clade 3 constitutes the lower stratigraphic layers of the site (layer 12 spit 6 – layer 16, spit 16).

The phylogeny constructed to include the PS samples is shown as a Bayesian tree (*Figure 2.6*). The PS data are denoted by coloured dates, derived from the stratigraphy of the site, modern (peach), 11,500 yr BP (blue), 15,200 yr BP (red) and 25,200 yr BP (green), data from TAW are coloured black. The resolution of the tree is poor in comparison to *Figure 2.5* due to the reduced sequence data utilised in this analysis (280 base pairs as opposed to 780). The 3 TAW clades

remain loosely associated, the PS data largely unresolved, but with some data associating within the TAW sequences.

Post phylogenetic analyses TAW sequence data were partitioned into the three clades identified in this study; sequence diversity parameters for these clades are shown in *Table 2.3*.

Table 2.3 *Sequence diversity parameters*

Samples	N	H	ti	tv	h	π
Clade 1	13	5	5	0	0.5385 (0.1611)	0.001134 (0.000951)
Clade 2	25	11	8	1	0.7333 (0.0952)	0.001338 (0.001026)
Clade 3	13	12	15	3	0.9872 (0.0354)	0.006377 (0.003728)

Molecular diversity indices (N = individuals, H = haplotypes, ti = transitions tv = transversions, h = haplotype diversity, π = nucleotide diversity, parentheses = standard error).

Figure 2.5 *Phylogeny for the arctic collared lemming from Trou Al'Wesse*
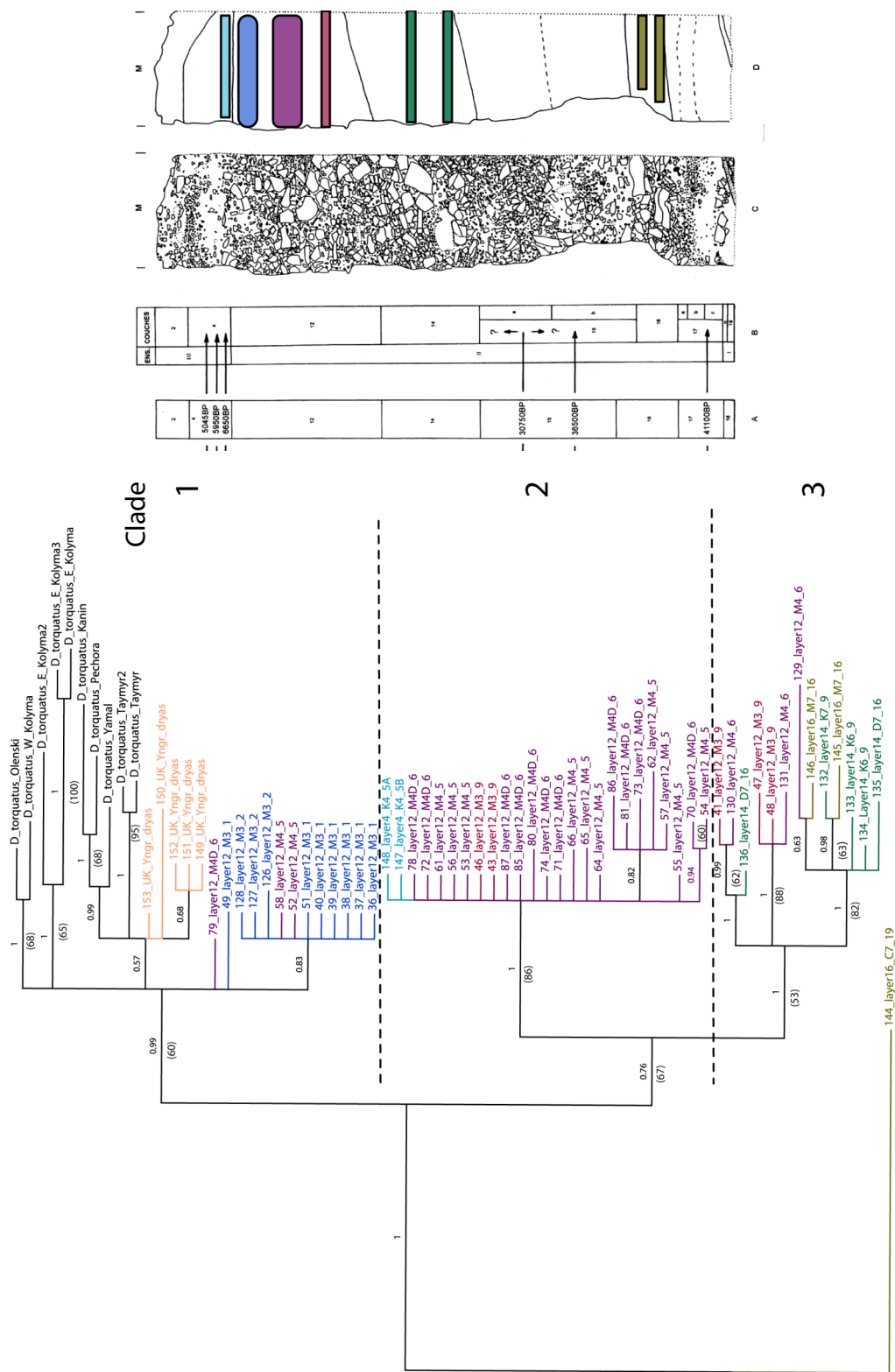
Bayesian tree constructed in MrBayes with HKY for 1000,000 generations. Bayesian probabilities are shown above the line and bootstrap values below the line in parentheses (only values above 50%). Sample identification number is followed by stratigraphic position. Colours represent stratigraphic position as shown in the column, samples in peach are from the UK, dating to the Younger Dryas period and those in black are modern sequences from Russia.

Figure 2.6 *Phylogeny for the arctic collared lemming including samples from Pymva-Shor (PS)*
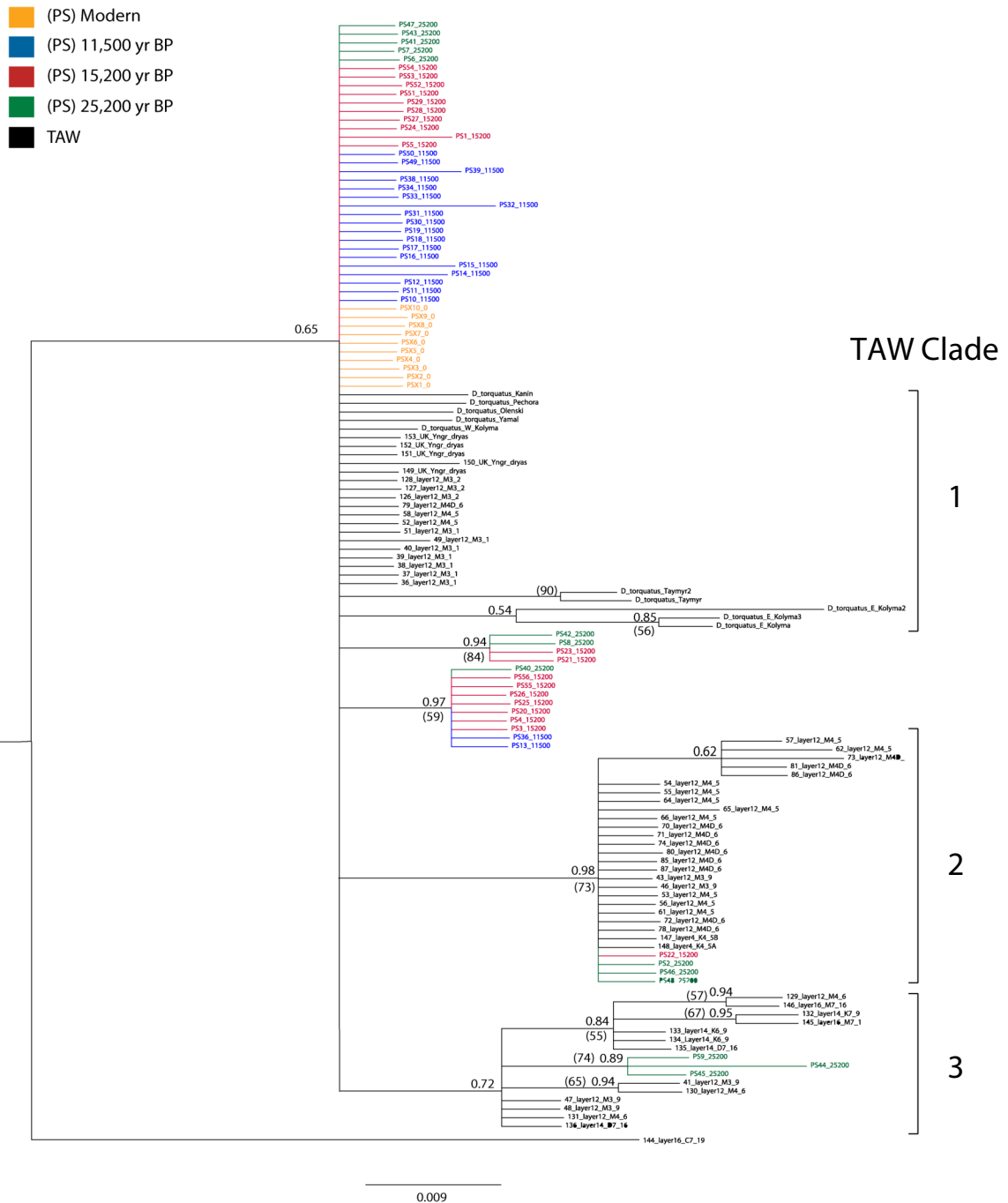
Bayesian tree constructed in MrBayes with HKY for 1000,000 generations. Nodal support is shown through approximate Bayesian probabilities above the line and bootstrap values (PAUP*) in parentheses (only values above 50% shown). Northern collared lemming used as an outgroup (not shown).

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2.4.3 Radiocarbon dating

From the 13 arctic collared lemming samples sent for radiocarbon dating, 8 were sufficiently well preserved to permit AMS dating. The remaining 5 samples failed due to low or no yield of collagen post the ultrafiltration process, thus there was insufficient well-preserved material for reliable dating. Successfully dated material were recorded in uncalibrated years BP and converted to calendar years with 95% confidence intervals using Oxcal v4.1 (Ramsey, 2009) with IntCal09 calibration curve (Reimer *et al.*, 2009) (*Table 2.4*).

Table 2.4 *Radiocarbon dated arctic collared lemming from Trou Al'Wesse, Belgium*

My ID	Layer	Square	Spit	Reference number	AMS 14C Date (yr BP)	Calibrated age range BP (95.4%)	Median (yr BP)	Clade
128	12	M3	2	OxA-2354-15	14415 ± 70	17865 - 17185	17539	1
51	12	M3	1	OxA-2352-39	15150 ± 120	18641 - 18021	18287	1
126	12	M3	2	OxA-2352-41	16980 ± 110	20443 - 19609	20166	1
52	12	M4	5	OxA-2354-14	17030 ± 90	20467 - 19876	20227	1
56	12	M4	5	OxA-2352-40	17780 ± 130	21535 - 20548	21230	2
130	12	M4	6	OxA-2354-16	22500 ± 190	27894 - 26327	27237	3
135	14	O7	16	OxA-2352-11	25650 ± 450	31101 - 29584	30449	3
131	12	M4	6	OxA-2352-42	26300 ± 380	31351 - 30356	30917	3
45	12	M3	9	Failed no yield				
133	14	K6	9	Failed no yield				
136	14	O7	16	Failed very low yield				
144	16	C7	19	Failed no yield				
146	16	M7	16	Failed very low yield				

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The arctic collared lemming dates obtained in this study follow a temporal pattern in accordance with the phylogeny identified, the youngest dated material grouping in clade 1, progressively ageing through clade 2 and into clade 3. Each clade had at least one dated sample, therefore timing of clade turnovers can be constrained to between the oldest and youngest date range identified from the AMS dated material. The timing of turnover event 1 (between clades 1 and 2) can be constrained to between 19,876 and 21,535 BP whilst turnover event 2 (between clades 2 and 3) occurred between 20,548 and 27,894 BP. Comparison between the temporal pattern identified from the TAW arctic collared lemmings and radiocarbon data sourced on mammoth are illustrated in *Figure 2.7*. The figure uses calibrated date ranges to compare the timing of arctic collared lemming clade turnover events, with mammoth occurrence on a regional European scale, and a localised scale, 1000 Km radius of TAW. *Figure 2.8* highlights the changes in climate during this period.

Figure 2.7 *Calibrated radiocarbon date ranges of the TAW arctic collard lemmings and mammoths*
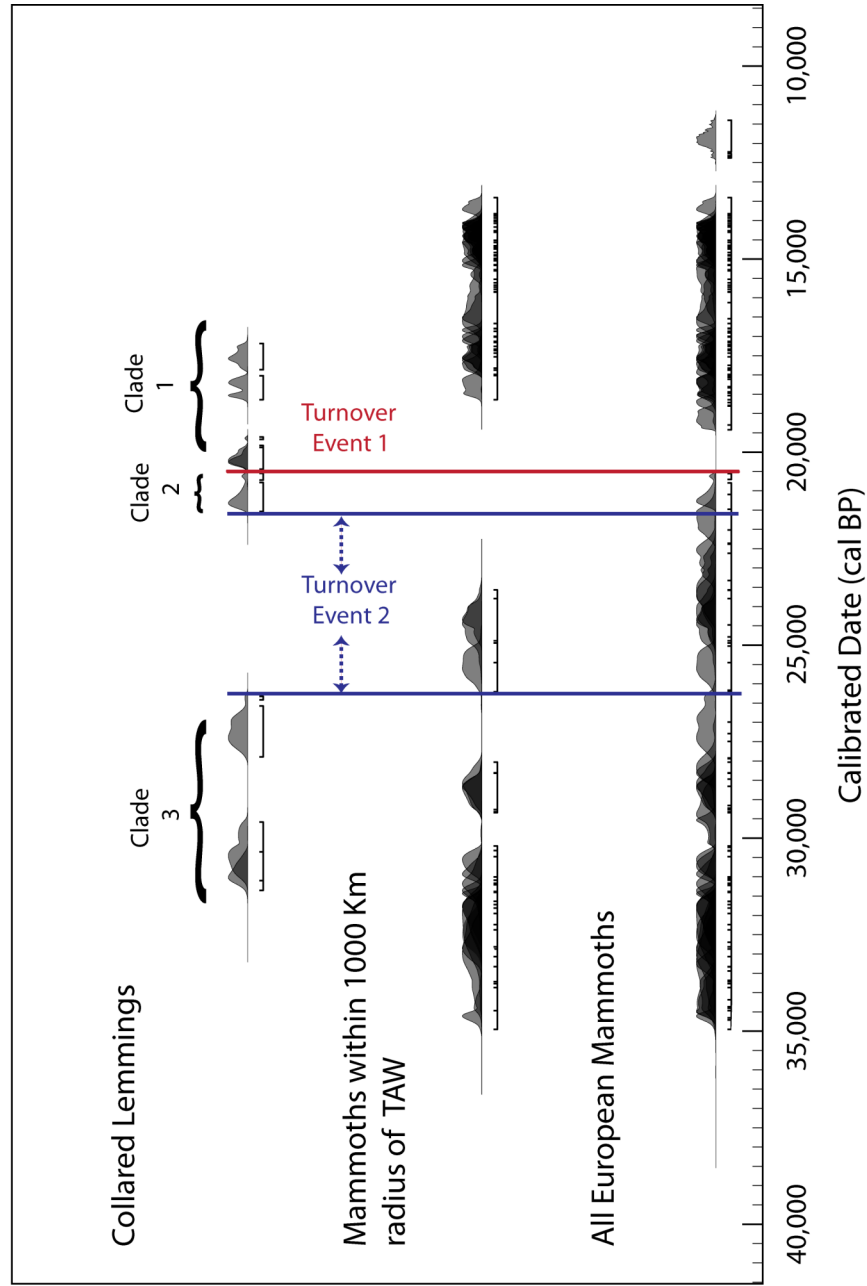
All calibrated date ranges were generated in Oxcal v4.1 using IntCal09 calibration curve

Figure 2.8 *Calibrated radiocarbon date ranges of the TAW arctic collard lemmings, mammoth (1000km) and climate change form the North Greenland Ice Core Project (NorthGRIP)*
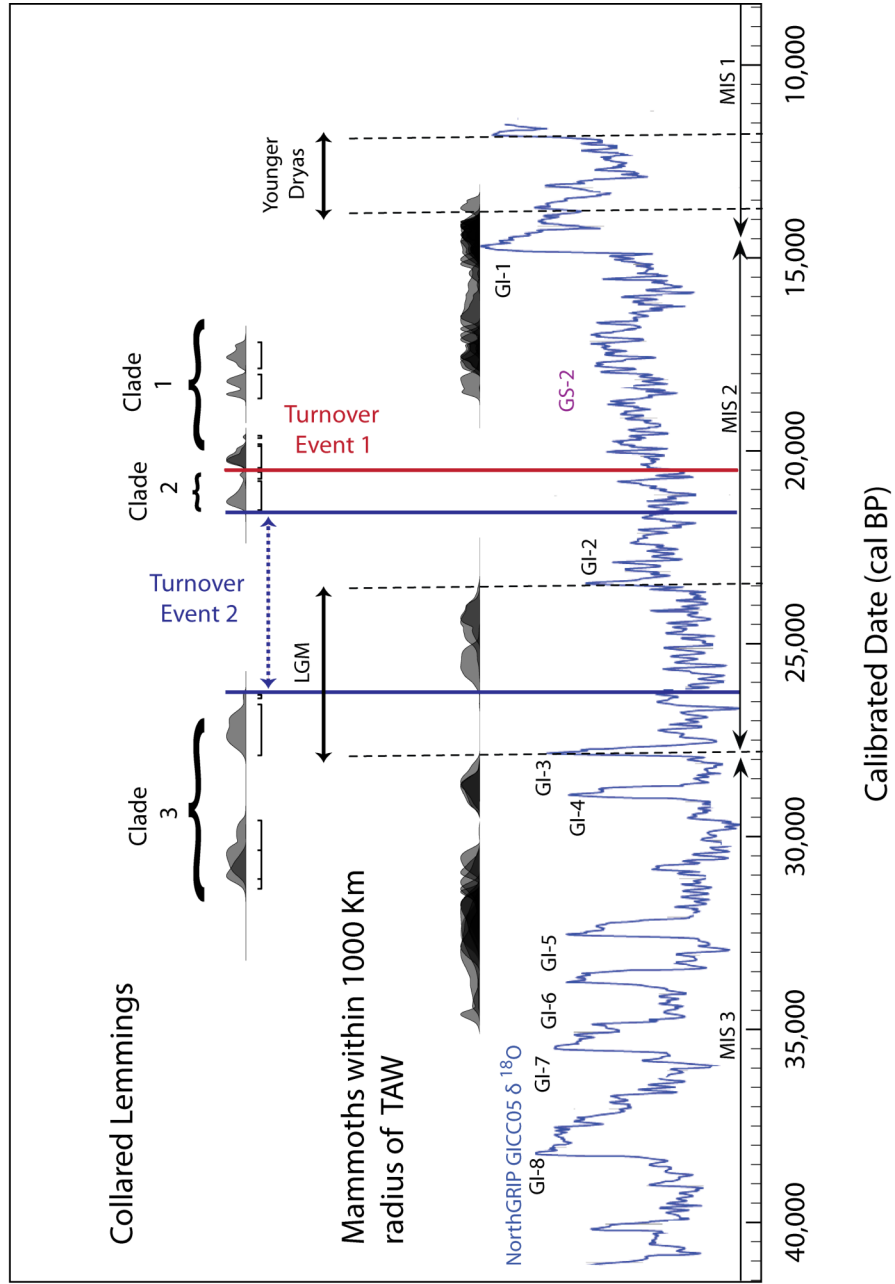
All calibrated date ranges were generated in Oxcal v4.1 using IntCal09 calibration curve NorthGRIP GICC05 data taken from Svensson et al. (2006) Abbreviations: MIS: Marine Isotopic Stages; GI: Greenland Interstadials; GS: Greenland Stadials

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2.5 Discussion

The phylogenetic structure revealed in this study identifies three genetically distinct lineages of arctic collared lemming (clades 1-3). There is also potential identification of a 4th clade, represented by a single sample (144). The sample was the sole sample from layer 16 spit 19 to successfully amplify mtDNA and stratigraphically represents the oldest sample in these analyses. It is therefore plausible that it represents an additional clade, however without additional samples to confirm this a conservative approach is taken, and reference will only be made in this discussion to the three main clades. Within the overall phylogeny, the stratigraphic location of samples approximately reflects lineage groupings, all samples below layer 12 group in clade 3, the majority of the middle of layer 12 form clade 2, while all samples from the top of layer 12 are found in clade 1. Anomalies to this pattern occur in layer 12 spits 5 and 6, where 79% of the total samples from this location occur in clade 2, but 10.5% fall in clade 1 and the remaining 10.5% in clade 3 and layer 12 spit 9 where samples occur in both clades 2 and 3.

Although relative dating of samples by stratigraphy is a valuable tool, direct AMS dating of samples is clearly both more accurate and provides an absolute estimate of the age of a sample. The samples directly dated in this study are distributed throughout the three lineages, although sample choice for dating was severely constrained by the amount of bone material available post extraction. The AMS dates obtained concord with both the over all stratigraphic pattern and the phylogenetic structure revealed in analyses; samples from the top layers of TAW (clade 1) are the youngest, the date from clade 2 follows on and samples from the lowest layers of the site (clade 3) generated the oldest dates. Interestingly, two of the samples (130 and 131) dated from clade 3 are from layer 12 spit 6, and as such are anomalous to the trend of stratigraphic location and lineage allocation, as the majority of spit 6 samples are found in clade 2. The AMS dates however, record them as far older, 6,000 and 9,200 years older than the dated sample in clade 2. It therefore seems likely that some reworking of material has occurred at this site. This does not reject the site stratigraphy as a whole, but rather highlights the importance of considering stratigraphic evidence, radiocarbon dates and

molecular phylogenies together, as opposed to relying solely on stratigraphic evidence, particularly when analysing small, easily disturbed materials. However, even when large bone material are under investigation, preferential selection for radiocarbon-dated material has been expressed, due to the potential movement of bones and ivory by early humans (Stuart *et al.*, 2002).

The partitioning of TAW samples into discrete temporal clades, allows comparative analyses of sequence diversity parameters through time. *Table 2.3* shows summary statistics of molecular diversity, calculated for each of the three clades. The results indicate a distinct decrease in diversity for all parameters through time, the oldest clade 3 exhibiting the greatest diversity, the youngest, clade 1 the least. This is consistent with findings from previous studies on ancient arctic collared lemming DNA diversity (Prost *et al.*, 2010) and modern populations (Fedorov *et al.*, 1999; Prost *et al.*, 2010) that exhibit a general decline in diversity throughout the Late Pleistocene, resulting in a relatively genetically depauperate population today. In the arctic collared lemming this has previously been attributed to population bottlenecks, driven by climatic change (Fedorov, Goropashnaya, 1999). Postglacial loss of genetic diversity is not unique to the arctic collared lemming. Hofreiter and Barnes (2010) identified that all ancient DNA studies of Late Pleistocene species extant in the northern Holarctic have observed significant loss in genetic diversity as the species move forward in time. The trend can be attributed to loss of genetic diversity during glacial maxima and insufficient time post the LGM for genetic diversity to have recovered to that observed prior to the LGM.

The phylogenetic evidence obtained in this study finds that TAW supported several lineages of arctic collared lemmings (characterised by mtDNA differences), which were separated into discrete time periods. This structure is indicative of genetic replacement at distinct periods in time. Mechanisms for this process could either be through outcompetition of the native group by the incoming group due to some selective advantage, genetic drift, and mutation or by local extinction of the native group, followed by replacement. There is no evidence to suggest that in this case, where the incoming group of arctic collared lemmings can be seen to be both genetically and morphologically quite similar to

the resident population, there would be any barrier to free interbreeding with the original population. Thus, even under circumstances where the incoming group possessed a selective advantage, it would generally pass this advantage on to the resident population through interbreeding. If selective advantage is disregarded, genetic drift or mutation can still result in genetic replacement. Time periods between genetic replacements at TAW are however far too limited (1 – 7 Kyr) for these to be viable. The remaining explanation for the genetic replacement observed is that the original population were extirpated, and an incoming population replaced the original with their own genetic signature. Considering the structure and timeline of events revealed in this analysis, two relatively fast genetic replacement events, I propose that population extinction followed by population recolonisation offers the most likely scenario to explain the data obtained.

Recolonisation of a region invokes the presence of a source population. TAW is on the southerly and westerly extent of arctic collared lemming distribution during the Late Pleistocene, and thus any source population would most likely have been located north and east of TAW. To test this theory an additional phylogeny was constructed using sequence data from TAW and a data set of both modern and ancient DNA of arctic collared lemmings from Pymva-Shor (PS) a site in the northern Pre-Urals (Prost *et al.*, 2010). The resultant phylogeny (*Figure 2.6*) effectively forms a large polytomy, with data associating into subclades. The resolution of the tree is poor, but support for the subclades is strong, providing validity for a degree of interpretation in these relationships. It is of particular interest that PS data from 15,000 and 25,200 yr BP group within the TAW subclades. The close association of PS samples from different time periods and TAW data is compatible with a source population. The haplotypes uncovered in the TAW phylogeny would have originated from a source population containing these and other haplotypes. Thus, when the source population is included in the analysis, sampling appears to occur essentially from a single large source population. This does not allow the conclusion that the arctic collared lemmings from TAW must be direct immigrants from the population in PS, but rather that the genetic association indicates that the source population is likely to have originated from a population associated with that at the PS site. To test the precise

location of the source would require extensive geographical sampling outside the aims of this study.

Most lemmings and voles particularly in the northern hemisphere are known to exhibit multiannual cyclic population density fluctuations - 'boom and bust' years - over a fairly regular periodicity of roughly 3-5 years (Elton, 1924; Ims, Fuglei, 2005; Stenseth, 1999). Although population decline during low phases can lead to loss of genetic diversity through the process of genetic drift, cyclic decline occurs over regular, short time periods and as such is unlikely to have caused the signal observed in this data, where the lineages identified span thousands of years. In addition, studies have indicated that cyclic density declines in lemming populations may be followed by periods of increased migration and gene flow, thus negating the effects of drift and work to stabilise genetic diversity (Ehrich, Jorde, 2005). It therefore seems likely that the pattern observed in this study is indeed indicative of population turnover events rather than reflecting shorter term 'boom and bust' cycles.

Direct dating of samples for each of the lineages allows an estimated timeframe for the observed turnover events. The most recent is estimated between 19,876 and 21,535 calibrated years BP the earlier between 20,548 and 27,894 calibrated years BP. The earliest period spans the last severe cold phase of the LGM (ca. 27 - 23ka cal), the later turnover occurs post LGM, but within the cold stage Greenland stadial 2 (ca. 23 – 14 Kyr BP) (*Figure 2.8*). It is possible that the severity of the LGM period may have become detrimentally harsh, resulting in localised extinction. It is also possible, that this reflects habitat and environmental change associated with the warmer Greenland Interstadial 3 estimated to 27,780 +/- 416 or Greenland Interstadial 2 estimated to 23,340 +/- 298 (Svensson *et al.*, 2006). A greater number of radiocarbon dates are obviously required to provide greater precision in determining the timing of turnover events, particularly for the earlier period. However, what is apparent from the available data is that during this dynamic cold phase, Marine Isotope Stage 2 (ca. 27 – 14 Kyr BP), the TAW population of arctic collared lemmings incurred two population turnover events.

The implications of this study can be further extended when utilised comparatively with data from another species. The woolly mammoth provides an interesting comparison, its habitat and climatic preferences are similar to that of the arctic collared lemming and there have accumulated an extensive number of radiocarbon dates to reconstruct spatiotemporal patterns of its disappearance (Barnes *et al.*, 2007; Lister, 2009; Sommer, Benecke, 2009; Stuart *et al.*, 2002). In order to contrast woolly mammoth with the TAW arctic collared lemmings European woolly mammoth radiocarbon dates were collated and designated as either European (general) or within close proximity (1000km) of TAW. Woolly mammoth are recorded as a continuous presence in Europe up to 30 Kyr BP and after 19 Kyr BP. The period in between has been associated with localised extinction and recolonisation.

To determine if arctic collared lemming population extinction and woolly mammoth absence in the TAW locale were correlated, the timing of the arctic collared lemming turnover events were plotted alongside the presence/absence of mammoth. The results (*Figure 2.7* and *2.8*) illustrate that the timing of the most recent turnover event in the arctic collared lemming (ca. 20 Kyr cal BP) corresponds to a gap in mammoth occurrence across Europe (21 – 19 Kyr cal BP). This hiatus in the mammoth record had previously been reported, although the cause of disappearance was unclear (Lister, Stuart, 2008; Stuart, 2005; Stuart *et al.*, 2004). The timing of the earlier turnover event in the arctic collared lemming (ca. 21 – 27 Kyr BP) corresponds to localised gaps in mammoth occurrence around the TAW area but not to the whole of Europe entire. Despite the broad temporal frame within which turnover 2 could have occurred (7 Kyr) only three 'local' mammoth dates are recorded during this entire period. In the absence of additional arctic collared lemming dating it is impossible to speculate as to whether these dates occur before, after or even during turnover 2. All correlation with mammoth data from the TAW region has the potential to suffer from insufficient data, however from the 66 European dates sourced only 26 are excluded from the localised 1000 Km zone. That 93% of the 'local' mammoth dates and 85% of the European dates occur outside the temporal frame of the combined turnover events (ca. 20 – 27 Kyr) remains a striking proportion. The validity of the mammoth data set to detect temporal hiatus is heightened through

the substantial accumulation of radiocarbon dates. While the arctic collared lemming data rely on a small number of dates to identify the timing of turnover events, it benefits from a phylochronological approach, extensively sampling from a stratified site to construct a well-supported phylogenetic representation of the data.

That the two turnover events revealed in this study correspond with a novel 'local' and a reported regional absence of mammoth is significant. Not only is it of interest that both small and large mammal species exhibit signs of localised population extinction in a corresponding spatiotemporal patterns, it also allows discussion of causation. The arctic collared lemming, a small mammal, would be an unlikely target for human hunting, and as such this would not be considered a viable explanation for localised extirpation. More feasible considerations would invoke climatic and/or environmental changes as fundamentally more probable. That the mammoth, a species with comparable habitat and environmental requirements exhibits a pattern of localised extinction within a similar timeframe is suggestive of an underlying causal event, such as one would expect from climatic and environmental change. While the NorthGRIP data do not indicate intense climatic change during the estimated timing of turnover events - excepting the potential association with either Greenland Interstadial 2 or 3 for the earliest turnover - (*Figure 2.8*). An alternative explanation would be that localised turnover in the arctic collared lemming and the mammoth represent an artefact of localised environmental change undetected through ice core data.

Both mammoth and arctic collared lemmings reoccupied parts of end-Pleistocene Europe (mammoth Western and Central Europe, arctic collared lemmings TAW), but repeat the pattern of local extinction, the mammoth ca. 14 Kyr BP, the last dated occurrence of the arctic collared lemming at TAW ca. 17 Kyr BP. But while the arctic collared lemming contracted northwards to its current habitat in the tundra of the high Arctic, the mammoth became globally extinct ca. 4 Kyr BP. The disparity could be contributed to the spatial requirements of the mammoth compared to those of the arctic collared lemming. Stewart *et al.* (2010) illustrate the relationship between size of the potential refugium and time to population extinction. Where the size requirements of the refugium are dictated by the spatial

requirements of the species, which are ultimately dependent on many factors, key of which include body size, generation length and trophic level.

The warming climate meant that both the arctic collared lemming and the mammoth were essentially returning to areas of polar refugia. Thus, the greater spatial requirements of the mammoth, combined with insufficient refugium size, may have contributed to its demise, conversely, the reduced spatial requirements of the arctic collared lemming, may have aided its survival. The contraction in range size may also have had increased prevalence for the mammoth with regards to 'extinction debt'. A concept whereby habitat loss or fragmentations, particularly for species with lower colonisation rates, that are efficient in resource utilisation, leads to vulnerability and deterministic extinction (Tilman *et al.*, 1994). The final extinction of the mammoth cannot therefore be correlated to data on the arctic collared lemmings from this study. The salient inference that can be gleaned from this investigation is that a small extant and a large extinct species appear to exhibit congruent patterns of local extinction during corresponding spatiotemporal periods, a pattern that is highly suggestive of size independent causation.

In summary, this study has sought to examine the historical pattern of an Arctic adapted species in a northern European environment. The pattern revealed is one of population extinction and recolonisation through time. That the timing of turnover events appears to correspond simultaneously with localised disappearance of the woolly mammoth favours a non selective explanation for MIS 2 extinction events and suggests the influential role of climatic and environmental change, through the identification of synchronous population extinctions of both micro and mega faunal species.

2.6 References

Alroy J (2001) A Multispecies Overkill Simulation of the End-Pleistocene Megafaunal Mass Extinction. *Science* **292**, 1893-1896.

Barnes I, Matheus P, Shapiro B, Jensen D, Cooper A (2002) Dynamics of Pleistocene Population Extinctions in Beringian Brown Bears. *Science* **295**, 2267-2270.

Barnes I, Shapiro B, Lister A*, et al.* (2007) Genetic Structure and Extinction of the Woolly Mammoth, *Mammuthus primigenius*. *Current Biology* **17**, 1072-1075.

Barnosky AD (1986) "Big game" extinction caused by late Pleistocene climatic change: Irish elk (Megaloceros giganteus) in Ireland. *Quaternary Research* **25**, 128-135.

Barnosky AD, Koch PL, Feranec RS, Wing SL, Shabel AB (2004) Assessing the Causes of Late Pleistocene Extinctions on the Continents. *Science* **306**, 70-75.

Conrad NJ, Bolus M (2003) Radiocarbon dating the appearance of modern humans and timing of cultural innovations in Europe: new results and new challenges. *Journal of Human Evolution* **44**, 331-371

Corbet GB (1978) *The mammals of the Palaearctic region: a taxonomic review* British Museum (Natural History) Cornell University Press.

Daulton TL, Pinter N, Scott AC (2010) No evidence of nanodiamonds in Younger-Dryas sediments to support an impact event. *Proceedings of the National Academy of Sciences* **107**, 16043-16047.

Diamond JM (1989) Quaternary megafaunal extinctions: Variations on a theme by paganini. *Journal of Archaeological Science* **16**, 167-175.

Ehrich D, Jorde PE (2005) High genetic variability despite high-amplitude population cycles in lemmings *Journal of Mammalogy* **86**, 380-385.

Elton CS (1924) Periodic Fluctuations in the Numbers of Animals: Their Causes and Effects. *J Exp Biol* **2**, 119-163.

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): An integrated software package for population genetics data analysis. Libertas Academica.

Faith JT, Surovell TA (2009) Synchronous extinction of North America's Pleistocene mammals. *Proceedings of the National Academy of Sciences* **106**, 20641-20645.

Fedorov, Fredga, Jarrell (1999) Mitochondrial DNA variation and the evolutionary history of chromosome races of collared lemmings (*Dicrostonyx*) in the Eurasian Arctic. *Journal of Evolutionary Biology* **12**, 134-145.

Fedorov VB (1999) Contrasting Mitochondrial DNA Diversity Estimates in Two Sympatric Genera of Arctic Lemmings (*Dicrostonyx*: *Lemmus*) Indicate Different Responses to Quaternary Environmental Fluctuations. *Proceedings: Biological Sciences* **266**, 621-626.

Fedorov VB, Goropashnaya AV (1999) The importance of ice ages in diversification of Arctic collared lemmings (*Dicrostonyx*): evidence from the mitochondrial cytochrome b region. *Hereditas* **130**, 301-307.

Ficcarelli G, Coltorti M, Moreno-Espinosa M*, et al.* (2003) A model for the Holocene extinction of the mammal megafauna in Ecuador. *Journal of South American Earth Sciences* **15**, 835-845.

Firestone BR (2009) The case for the Younger Dryas extraterrestrial impact event: Mammoth, Megafauna, and Clovis Extinction, 12,900 Years Ago. . *The Journal of Cosmology* **2**, 256-285.

Firestone RB, West A, Kennett JP*, et al.* (2007) Evidence for an extraterrestrial impact 12,900 years ago that contributed to the megafaunal extinctions and the Younger Dryas cooling. *Proceedings of the National Academy of Sciences* **104**, 16016-16021.

Gaudzinski S, Turner E, Anzidei AP*, et al.* (2005) The use of Proboscidean remains in every-day Palaeolithic life. *Quaternary International* **126-128**, 179-194.

Gowlett JAJ, Hedges REM, Law IA, Perry C (1986) Radiocarbon dates from the oxford ams system: archaeometry datelist 4. *Archaeometry* **28**(2), 206-221.

Gowlett JAJ, Hedges REM, Law IA, Perry C (1987) Radiocarbon dates from the oxford ams system: archaeometry datelist 4. *Archaeometry* **29**(1), 125-155.

Grayson D (1984) Nineteenth-Century Explanations of Pleistocene Extinctions: A Review and Analysis. In: *Quaternary Extinctions: A Prehistoric Revolution* (eds. Martin PS, Klein RG), pp. 5 - 39. The University of Arazona Press.

Grayson DK (2007) Deciphering North American Pleistocene Extinctions. *Journal of Anthropological Research* **63**, 185-214.

Grayson DK (2008) Holocene underkill. *Proceedings of the National Academy of Sciences* **105**, 4077-4078.

Grayson DK, Meltzer DJ (2003) A requiem for North American overkill. *Journal of Archaeological Science* **30**, 585-593.

Guthrie RD (2004) Radiocarbon evidence of mid-Holocene mammoths stranded on an Alaskan Bering Sea island. *Nature* **429**, 746-749.

Haile J, Froese DG, MacPhee RDE*, et al.* (2009) Ancient DNA reveals late survival of mammoth and horse in interior Alaska. *Proceedings of the National Academy of Sciences* **106**, 22352-22357.

Hasegawa M, Kishino H, Yano T-a (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **22**, 160-174.

Hedges REM, Housley RA, Law IA, Perry C (1988) Radiocarbon dates from the Oxford AMS system: archaeometry datelist 7. *Archaeometry* **30**(1), 155-164.

Hedges REM, Housley RA, Law IA, Bronk CR (1989) Radiocarbon dates from the Oxford AMS system: Archaeometry Datelist 9. *Archaeometry* **31**(2), 207-234.

Hedges REM, Housley RA, Bronk CR Van Klinken GJ (1996) Radiocarbon dates from the Oxford AMS system: archaeometry datelist 25. *Archaeometry* **38**(1), 187-207.

Hedges REM, Pettitt PB, Bronk CR, Van Klinken GJ (1997) Radiocarbon dates from the Oxford AMS system: archaeometry datelist 25. *Archaeometry* **39**(1), 247-262.

Hedges REM, Pettitt PB, Bronk CR, Van Klinken GJ (1998) Radiocarbon dates from the Oxford AMS system: archaeometry datelist 25. *Archaeometry* **40**(1), 227-239.

Hofreiter M, Barnes I (2010) Diversity lost: are all Holarctic large mammal species just relict populations? *BMC Biology* **8**, 46.

Ims RA, Fuglei EVA (2005) Trophic Interaction Cycles in Tundra Ecosystems and the Impact of Climate Change. *BioScience* **55**, 311-322.

Kennett DJ, Kennett JP, West A*, et al.* (2009) Nanodiamonds in the Younger Dryas Boundary Sediment Layer. *Science* **323**, 94.

Jacobi RM, Higham TFG (2009) The early Lateglacial re-colonization of Britain: new radiocarbon evidence from Gough's Cave, southwest England. *Quaternary Science Reviews* **28**(19-20), 1895-1913.

Koch PL, Barnosky AD (2006) Late Quaternary Extinctions: State of the Debate. *Annual Review of Ecology, Evolution, and Systematics* **37**, 215-250.

Krause J, Dear PH, Pollack JL*, et al.* (2006) Multiplex amplification of the mammoth mitochondrial genome and the evolution of Elephantidae. *Nature* **439**, 724-727.

Kuzmin VY (2009) Extinction of the woolly mammoth (*Mammuthus primigenius*) and woolly rhinoceros (*Coelodonta antiquitatis*) in Eurasia: Review of chronological and environmental issues. *Boreas* **39**, 247-261.

Lister AM (2009) Late-glacial mammoth skeletons (*Mammuthus primigenius*) from Condover (Shropshire, UK): anatomy, pathology, taphonomy and chronological significance. *Geological Journal* **44**, 447-479.

Lister AM, Stuart AJ (2008) The impact of climate change on large mammal distribution and extinction: Evidence from the last glacial/interglacial transition. *Comptes Rendus Geosciences* **340**, 615-620.

Lõugas L, Ukkonen P, Jungner H (2002) Dating the extinction of European mammoths: new evidence from Estonia. *Quaternary Science Reviews* **21**, 1347-1354.

Lyons SK, Smith FA, Wagner PJ, White EP, Brown JH (2004) Was a 'hyperdisease' responsible for the late Pleistocene megafaunal extinction? *Ecology Letters* **7**, 859-868.

Lyons SKS, F. A. Brown, J. H. (2004) Of mice, mastodons and men: human-mediated extinctions on four continents. *Evolutionary Ecology Research* **6**, 339-358.

MacPhee RDE, Marx P (1997) The 40,000-year plague: Humans, hyperdisease, and first-contact extinctions. In: *Natural Change and Human Impact in*

Madagascar (eds. Goodman SM, Patterson BD), pp. 169 - 217. Smithsonian institution Press.

Martin PS (1966) Africa and Pleistocene Overkill. *Nature* **212**, 339-342.

Martin PS (1973) The Discovery of America. *Science* **179**, 969-974.

Martin PS (1984) Prehistoric overkill: the global model. In: *Quaternary Extinctions: A Prehistoric Revolution* (eds. Martin PS, Klein RG), pp. 354 - 403. University of Arizona Press, Tuscon.

Maul LC, Markova AK (2007) Similarity and regional differences in Quaternary arvicolid evolution in Central and Eastern Europe. *Quaternary International* **160**, 81-99.

Miller R, Stewart J, Zwyns N, Otte M (2009) The stratified early to late mesolithic sequence at Trou Al'Wesse (Modave, Belgium). In: *Chronology and Evolution within the Mesolithic of North-West Europe: Proceedings of an International Meeting, Brussels, May 30th-June 1st 2007* (eds. Crombé P, Van Strydonck M, Sergant J, Boudin M, Bats M), pp. 287-306. Cambridge Scholars Publishing, Newcastle upon Tyne.

Nadachowski A (1982) *Late quaternary rodents of poland with special reference to morphotype dentition analysis of voles* Polska Akademia Nauk, Kraków.

Pirson S (1999) Étude sédimentologique préliminaire au Trou Al'Wesse (Modave, Belgique). *Bulletin de la Société royale belge d'études géologiques et archéologiques (Les Chercheurs de la Wallonie)* **XXXIX**, 115 - 162.

Posada D, Crandall K (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817-818.

Prost S, Smirnov N, Fedorov VB, *et al.* (2010) Influence of Climate Warming on Arctic Mammals? New Insights from Ancient DNA Studies of the Collared Lemming Dicrostonyx torquatus. *PLoS one* **5**, e10447.

Pushkina D, Raia P (2008) Human influence on distribution and extinctions of the late Pleistocene Eurasian megafauna. *Journal of Human Evolution* **54**, 769-782.

Ramsey B, C. (2009) Bayesian analysis of radiocarbon dates. *Radiocarbon* **51**, 337-360.

Reimer PJ, Baillie MGL, Bard E*, et al.* (2009) INTCAL09 AND MARINE09 radiocarbon age calibration curves, 0-50,000 years cal BP. *Radiocarbon* **51**, 1111-1150.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572-1574.

Scott AC, Pinter N, Collinson ME*, et al.* (2010) Fungus, not comet or catastrophe, accounts for carbonaceous spherules in the Younger Dryas "impact layer". *Geophys. Res. Lett.* **37**, L14302.

Scourse JD, Coope GR, Allen JRM, Lister AM, Housley RA, Hedges REM, Jones ASG, Watkins R (2009) Late-glacial remains of woolly mammoth (*Mammuthus primigenius*) from Shropshire, UK: stratigraphy, sedimentology and geochronology of the Condover site. *Geological Journal* **44**, 392-413.

Shapiro B, Drummond AJ, Rambaut A*, et al.* (2004) Rise and Fall of the Beringian Steppe Bison. *Science* **306**, 1561-1565.

Sommer RS, Benecke N (2009) First radiocarbon dates on woolly mammoth (*Mammuthus primigenius*) from northern Germany. *Journal of Quaternary Science* **24**, 902-905.

Stenseth NC (1999) Population Cycles in Voles and Lemmings: Density Dependence and Phase Dependence in a Stochastic World. *Oikos* **87**, 427-461.

Stevens RE, O'Connell TC, Hedges REM, Street M (2009) Radiocarbon and stable isotope investigations at the Central Rhineland sites of Gönnersdorf and Andernach-Martinsberg, Germany. *Journal of Human Evolution* **57**(2), 131-148.

Stewart JR, Lister AM, Barnes I, Dalén L (2010) Refugia revisited: individualistic responses of species in space and time. *Proceedings of the Royal Society B: Biological Sciences* **277**, 661-671.

Stewart JR, van Kolfschoten, M., Markova, A. and Musil, R. (2003) The Mammalian Faunas of Europe during Oxygen Isotope Stage Three. In: *Neanderthals and Modern Humans in the European Landscape during the Last Glaciation, 60,000 to 20,000 years ago: Archaeological Results of the Stage 3 Project* (ed. Andel THv), pp. 103-129. McDonald Institute Monograph Series.

Stuart AJ (2005) The extinction of woolly mammoth (Mammuthus primigenius) and straight-tusked elephant (Palaeoloxodon antiquus) in Europe. *Quaternary International* **126-128**, 171-177.

Stuart AJ, Kosintsev PA, Higham TFG, Lister AM (2004) Pleistocene to Holocene extinction dynamics in giant deer and woolly mammoth. *Nature* **431**, 684-689.

Stuart AJ, Sulerzhitsky LD, Orlova LA, Kuzmin YV, Lister AM (2002) The latest woolly mammoths (Mammuthus primigenius Blumenbach) in Europe and Asia: a review of the current evidence. *Quaternary Science Reviews* **21**, 1559-1569.

Svensson A, Andersen KK, Bigler M*, et al.* (2006) The Greenland Ice Core Chronology 2005, 15-42†ka. Part 2: comparison to other records. *Quaternary Science Reviews* **25**, 3258-3267.

Swofford DL (2000) Phylogenetic analysis using parsimony (* and other methods),. Sinaur Associates, Sunderland, Massachusetts.

Tilman D, May RM, Lehman CL, Nowak MA (1994) Habitat destruction and the extinction debt. *Nature* **371**, 65-66.

Ugan A, Byers D (2008) A global perspective on the spatiotemporal pattern of the Late Pleistocene human and woolly mammoth radiocarbon record. *Quaternary International* **191**, 69-81.

Ukkonen P, Arppe L, Houmark-Nielsen M, Kjêr KH, Karh JA (2007) MIS 3 mammoth remains from Sweden--implications for faunal history, palaeoclimate and glaciation chronology. *Quaternary Science Reviews* **26**, 25-28.

Ukkonen P, Lunkka JP, Jungner H, Donner J (1999) New radiocarbon dates from Finnish mammoths indicating large ice-free areas in Fennoscandia during the Middle Weichselian. *Journal of Quaternary Science* **14**, (7), 711-714.

Vartanyan SL, Arslanov KA, Karhu JA, Possnert G, Sulerzhitsky LD (2008) Collection of radiocarbon dates on the mammoths (*Mammuthus primigenius*) and other genera of Wrangel Island, northeast Siberia, Russia. *Quaternary Research* **70**, 51-59.

Veltre DW, Yesner DR, Crossen KJ, Graham RW, Coltrain JB (2008) Patterns of faunal extinction and paleoclimatic change from mid-Holocene mammoth

and polar bear remains, Pribilof Islands, Alaska. *Quaternary Research* **70**, 40-50.

Veness C (2002-2010) Calculate distance, bearing and more between Latitude/Longitude points.http://www.movable-type.co.uk/scripts/latlong.html

Wroe S, Field J, Fullagar R, Jermin LS (2004) Megafaunal extinction in the late Quaternary and the global overkill hypothesis. *Alcheringa: An Australasian Journal of Palaeontology* **28**, 291 - 331.

Yang DY, Eng B, Waye JS, Dudar JC, Saunders SR (1998) Improved DNA extraction from ancient bones using silica-based spin columns. *American Journal of Physical Anthropology* **105**, 539-543.

Chapter 3: Population study of the European water vole

3.1 Abstract

The oscillating climate of the late Quaternary has led to widespread faunal extinction and translocation. Periodic glacial advancement would have made many parts of Europe uninhabitable for temperate species over thousands of years. Molecular studies of extant species have been extensively utilised to assess where temperate species survived and how they repopulated Europe post the last glaciation. Here I demonstrate the advantages of an ancient DNA approach to unravel postglacial histories of the water vole (*Arvicola amphibius*, formerly *A. terrestris*). In the UK, modern data had previously identified molecular distinction between English and Scottish water voles, but was unable to detect details of events prior to this outcome. I obtained Pleistocene, early Holocene and additional modern water vole samples from across the UK and Europe. Phylogenetic analysis, using mitochondrial DNA (control region), reveals that two temporally distinct colonisation events occurred in the UK. An initial water vole lineage was replaced in England by a second lineage ca. 12 – 8 Kyr BP. The replacement event was however limited to England; the first colonising lineage therefore remains, but is restricted to Scotland. In addition, I examine European postglacial colonisation events, specifically the strict refugial model, where northern Europe is repopulated exclusively from refugia populations in the southern peninsulas. The inclusion of ancient samples from across Europe reveals genetic contribution of pre glacial, non-refugial populations, thus highlighting that current northern European populations are not exclusively derived from southern refugia populations.

3.2 Introduction

The later Quaternary, has been dominated by extensive climatic and environmental change, with cycles of glacial advance and retreat throughout. This has led to vegetational change and widespread faunal extinction and translocation. Across Eurasia, ice sheets periodically spread down from the north, leaving

Northern Europe almost fully glaciated and permafrost extending throughout Central Europe, essentially leaving only the southernmost peninsular ice free (Svendsen *et al.*, 2004) (*Introduction Section 1.5 Europe, Figure 1.4).*

The pattern of glacial advance and retreat occurred repeatedly throughout the dynamic climatic oscillations of the later Quaternary. The process created shifting regions of suitable habitat for both fauna and flora. During glacial advancement, habitats suitable for temperate species were compressed towards the south, to beyond the tundra on the margins of the permafrost. As the ice sheets gradually retreated temperate habitat expanded northwards in its wake. The retreat of the last glaciation has been considered instrumental in shaping the phylogeographic structure of species found in the northern hemisphere today (Hewitt, 1996; 1999; 2000).

Haffer (1969) conceived the term 'refuge areas', with reference to an isolated habitat associated with climatic change. In a study of the Amazon rainforest, he proposed that dry climatic periods of the Pleistocene would result in an increase in savannah environments, with only small separated pockets of forested areas suitable to provide refuge for forest dwelling animals. Periods of isolation would be followed by unification as climate altered, the repeated process of which driving population differentiation, ultimately resulting in the high diversity observed in the Amazon forest today.

Temperate European fauna are thought to have been restricted to southern refugia beyond the ice-permafrost boundary: the Iberian, Italian and Balkan peninsulas. Hewitt (1996; 1999; 2000) has made significant developments in the identification of the European refugial model, proposing that temperate populations survived glacial periods within these three refugial peninsulas, swiftly expanding north as glaciers retreated. This rapid response of species in following suitable habitat, 'habitat tracking' had already been proposed by Eldredge (1989; 1995). Eldredge formulated 'habitat tracking' to describe species response to environmental change, suggesting that rather than constantly evolving to adapt to new environments, species would instead, search for and move towards familiar habitat. This provided an alternative to gradual evolutionary changes, and an

underlying explanation for the high level of stasis observed, across species, in the fossil record. While the generality of this proposal has been called into question by work on the Arctic fox (*Alopex lagopus*) (Dalén *et al.*, 2007), it remains the most plausible explanation of recolonisation dynamics available.

The phylogenetic evidence found by Dalén *et al.* (2007) suggest that post-glacial Scandanavia was recolonised by Arctic fox from northeastern Siberia, as opposed to Pleistocene Artic fox habitat tracking from Europe. If the Siberian population were the initial recolonisers, Arctic fox dispersing from Europe would have arrived in an already colonised region. Under the Hewitt (1996) model of post-glacial recolonisation, the initial recolonisers possess the greatest advantage. The model dictates that a small subset of long distance dispersers would colonise any newly available habitat ahead of the main distribution. These early populations would flourish due to resource availability, thus a small subset would rapidly establish and dominate the gene pool. The migrants that followed would arrive at an area with an established population at carrying capacity, and therefore provide only a limited contribution to the genetic structure of the population. These continuous founder events resulted in repeated bottlenecks, loss of alleles and increased homozygosity, which would be apparent in modern populations. In contrast, extant populations in areas that formerly acted as refugium would be expected to exhibit a greater genetic variability.

However, Bilton *et al.* (1998) observed that greater genetic variability does not necessarily indicate a refugium site. He proposed that the increased genetic variability in the Mediterranean peninsulas were indicative of endemism, through mutation accumulation and allopatric speciation. These were heightened in the Mediterranean peninsulas due to prolonged periods of geographic isolation, when, particularly for smaller mammals, mountainous regions limit dispersal during interglacial as well as glacial cycles. Stewart *et al.* (2010) note that if a species never expands from a region, then that region should not be defined as a refugium, concurring that a location continuously inhabited by a species through at least one full glacial and interglacial cycle would exhibit the greatest level of genetic diversity.

The genetic signature of a population bottleneck has, however, been found in many European species, often attributed to expansion from the proposed Iberian, Italian and Balkan refugia; these include not only mammal species, but also birds (Brito, 2005); insects (Lunt *et al.*, 1998); and amphibians (Martínez-Solano *et al.*, 2006). Hewitt (1999) describes three generic patterns of post-glacial northerly recolonisation, typified by the grasshopper (*Chorthippus parallelus*), hedgehog (*Erinaceus europeus/concolor*) and bear (*Ursus arctos*) (*Figure 3.1*) and further reviewed with additional studies (Hewitt, 2001).

Figure 3.1 *Schematic of the three generic patterns of post-glacial northerly recolonisation*

| *Chorthippus parallelus* | *Erinaceus spp* | *Ursus arctos* |
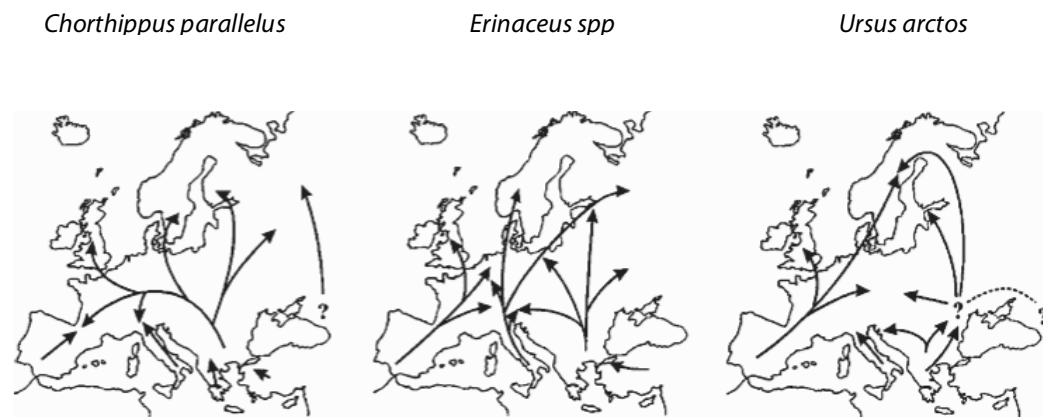
The base of the arrows indicate the location of a refugium, the direction indicates the proposed recolonisation route. Taken from Hewitt (1999)

The grasshopper was proposed to have repopulated Europe almost exclusively from a Balkan refuge, the Iberian and Italian colonisers contained by the Pyrenees and the Alps; the hedgehog was thought to have expanded into Europe from all three refugia; and the bear were proposed to have repopulated Europe from Iberia and the Balkans, effectively restricting the expansion of the Italian population.

Although an elegant explanation of the patterns observed in European fauna and flora, the three refugia model has been challenged, and additional refugia sites proposed. Stewart and Lister (2001) advocate locations north of the Mediterranean, 'cryptic northern refugia' (CNR), citing the potential of valley located caves, such as Kent's Cavern in Devon (UK), where temperate mammal fossils have been found in layers associated with glacial periods. They propose

that sheltered valleys provided microclimates for temperate survival, from where northern repopulation could have occurred. Willis (2000) found evidence of cold intolerant trees across Hungary throughout the last glaciation, implying a suitable microclimate in these areas. Further support for CNR comes from work by Kotlík and colleagues (2006) on the origins of the bank vole (*Clethrionomys glareolus*), a woodland vole with a wide European distribution including Iberian, Spain and the Balkans. Using mitochondrial DNA (mtDNA) they identified a monophyletic 'Carpathian clade', suggesting a more complex mode of recolonisation from multiple refugia sites, including one from the Carpathians. A further study incorporated museum skins to enable greater geographical coverage confirmed the Carpathians as a refugium for bank voles (Wójcik *et al.*, 2010). The study went on to identify a widespread occurrence of the Carpathian genetic signature, suggesting broad geographic expansion of the bank vole from a Carpathian refugium. In general, recent phylogeographic studies have highlighted the existence of a number of new or unconfirmed 'cryptic' refugia (Provan, Bennett, 2008).

The utilisation of ancient DNA in reconstructing species' histories has played a significant role in identifying source populations and postglacial recolonisation events. Valdiosera *et al.* (2007) analysed ancient DNA from European brown bear (*Ursus arctos*), according to Hewitt (1999) results should have indicated population isolation; instead, the ancient DNA revealed high levels of genetic diversity outside of the proposed refugia, with mixed haplotypes across regions. This indicates that continuous gene flow occurred across Southern Europe and that postglacial expansion was not restricted to an isolated refugium. A further study focusing on Iberian brown bear fossil DNA identified a dynamic pattern of continuous gene flow, devoid of genetic isolation (Valdiosera *et al.*, 2008).

In this study I utilise an ancient DNA perspective to examine population history of the Northern water vole (*Arvicola amphibius,* formerly *A. terrestris*). The Northern water vole is a widely distributed species found across Europe (excluding Ireland and Central and Southern Spain), east through Siberia to the Lena River Basin; from the Arctic Sea south to Lake Baikal and North West

China through North West Iran, Iraq, North Israel, Caucasus and Turk (Musser, Carleton, 2005).

The taxonomy of the water vole genus has been consistently varied, ranging from one all encompassing species (*terrestris*) Ellerman and Morrison-Scott (1951), more commonly two (*sapidus* and *terrestris*) Corbet *et al.* (1970), but also four (*amphibius*, *sapidus*, *scherman* and *terrestris*) Hinton (1926) and at its zenith seven (*amphibius*, *illyricus*, *italicus*, *musignani*, *sapidus*, *scherman* and *terrestris*) Miller (1912). Current taxonomy recognises three species *A. amphibius*, (Northern water vole, distributed across Eurasia), *A. sapidus* (Portugal, Spain and France) and *A. scherman* (European mountains: Alps, Carpathians, Cantabrian, Massif Central and Pyrenees) (Musser, Carleton, 2005), with a caveat that authors anticipate convergence towards the greater diversity recognised by Miller (1912).

Further complexity associated with Northern water vole nomenclature is derived from Linnaeus' (1758) proposal of two species, appearing on the same page, on the same work, *amphibius* and *terrestris*. The two forms have for some time been considered conspecific, with the binomial *terrestris* in common usage (Jefferies *et al.*, 1989; Lawton, Gordon, 1991; Woodroffe *et al.*, 2008), probably due to the original line priority afforded by Linneaus to *terrestris* (Corbet *et al.*, 1970). However, in the strictest taxonomic terms nomenclature is denoted by the first revisor to consider the species conspecific, Blasius (1857), who employed *amphibius*, placing *terrestris* as the subjective synonym, hence some authors preferred to use *amphibius* (Ashby *et al.*, 1969; Perry, 1943). Attempts to remove this ambiguity have been made through recent reversion, defaulting to the rigorously correct *A. amphibius* (Musser, Carleton, 2005). However, to date this has not been welcomed or upheld by all authors (Ventura, Casado-Cruz, 2010).

Several studies have sought to resolve the water vole lineage through molecular analyses. An mtDNA study by Taberlet *et al* (1998) identified two species and four lineages of water vole; *A. sapidus*, and 3 lineages or subspecies of *A. terrestris*; *A. t. italicus* (Italy), an aquatic form, *A. t. terrestris* (Northern and Central Europe) and a fossorial form, *A. t. scherman* (Northern Spain, Pyrennes, Massif Central and the Alps). The observed lineages were viewed as positive

evidence for postglacial recolonisation from Iberian, Italian and Balkan refugia and were included in a later paper as further evidence for the southern peninsular refugial model, the water vole described as a 'bear type' recoloniser (Hewitt 1999).

A further mtDNA study, specifically targeted water vole from across the United Kingdom (UK) (Piertney *et al.*, 2005). The resultant phylogeny identified the presence of two distinct clades, one with haplotypes from England/Wales the second with haplotypes from Scotland. A geographic and genetic division of this nature suggests that two colonisation events occurred in the UK. Inference from the within clade association of 5 representative European samples, highlighted that the Scottish population was derived from an Iberian population, and the English/Welsh population from Eastern Europe. However, due to the limitations of an exclusively modern DNA based dataset, it was not possible to discern whether the two colonisation events were separated geographically, but occurred at the same time, or, whether events were temporally distinct, the second colonisers replacing the first, in one or other of the geographical regions.

The application of an ancient DNA approach therefore provided an ideal opportunity to explore water vole colonisation of the UK. Through the inclusion of Pleistocene, early Holocene and additional modern water vole samples, I focus on resolving the details surrounding water vole postglacial colonisation of the UK. I explore the mode of colonisation, discerning whether events were geographically or temporally separated and the timing of this event or events. In addition to the UK material, I incorporate additional European samples, and assess the applicability of the southern peninsula refugia model to explain water vole postglacial colonisation events. Finally, although not the focus of this study, the addition of across species samples affords a molecular perspective on water vole taxonomy.

3.3 Materials and methods

3.3.1 Samples

A total of 82 water vole samples were collected from across Europe (*Figure 3.2 & Table 3.1*). Sample choice was restricted by availability of material for destructive purposes, but aimed to source material from the Late Pleistocene through to modern. The UK and surrounding areas were of highest priority, but sampling, particularly for modern materials, extended throughout Europe to provide a widespread reflection of current European haplotypes. Modern samples were obtained from archived museum sources, collected within the last 100 years. Mark Ruddy (Geography, RHUL) collected the majority of the ancient samples as part of the Ancient Human Occupation of Britain II project (AHOBII). Materials utilised were mandibles, identified through morphological characteristics, species level identifications were conducted by source museums (*Table 3.1*).

Figure 3.2 *Map of water vole sample sites*
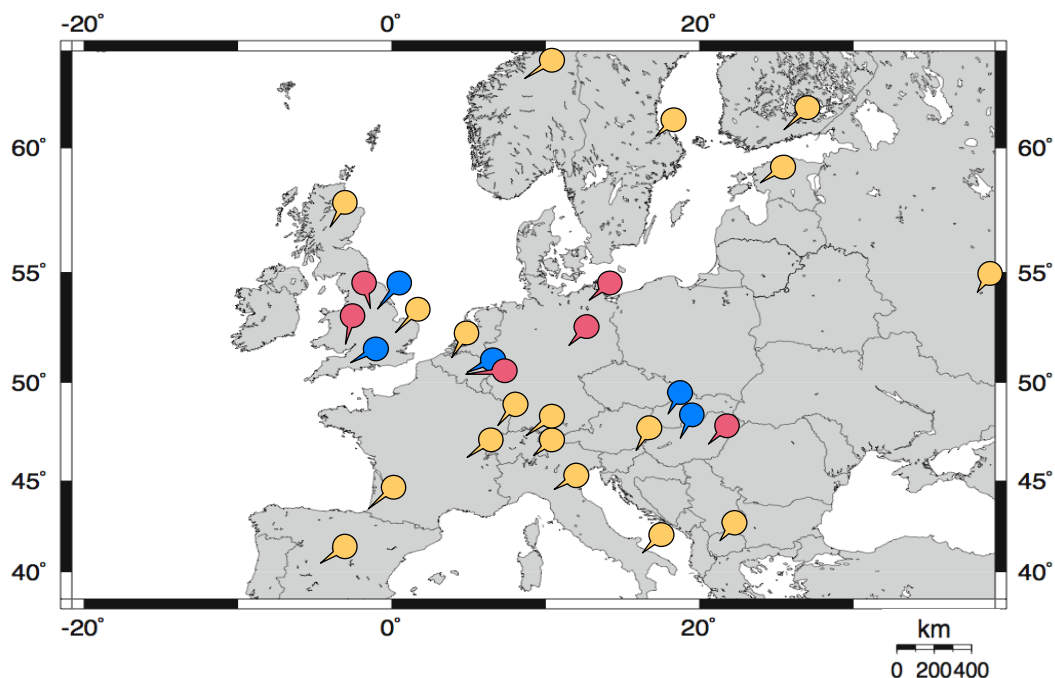
Samples from the Pleistocene are coloured blue; Holocene samples pink; and modern samples yellow

Table 3.1 *Water vole samples*

Species	Location	Site	Approximate Age	Number of Samples	Source
A. amphibius	Belgium, Ardennes	Trou Al'Wesse	Holocene	1	J. Stewart (BU)
A. amphibius	Belgium, Ardennes	Trou Al'Wesse	Pleistocene	4	J. Stewart (BU)
A. amphibius	Belgium, Ardennes	Trou du Frontal	Younger Dryas	1	RBINS [MR]
A. amphibius	Belgium, Namur	Marie-Jeanne Cave	Early Holocene	1	RBINS [MR]
A. amphibius	Belgium, Namur	Marie-Jeanne Cave	Pleistocene	3	RBINS [MR]
A. amphibius	England, Somerset	Bridged Pot	Younger Dryas	5	J. Stewart (BU)
A. amphibius	England, Somerset	Bridged Pot	Younger Dryas	1	NHM (London) [MR]
A. amphibius	England, Somerset	Gully Cave	Younger Dryas	1	D. Shreeve (RHUL)
A. amphibius	England, Staffordshire	Ossom's Eyrie Cave	Roman	2	MUM [MR]
A. amphibius	England, Stafforshire	Thor's Fissure	Roman	1	Buxton Museum [MR]
A. amphibius	England, Derbyshire	Pin Hole Lower Cave	Pleistocene	3	MUM [MR]
A. amphibius	England, Derbyshire	Wigber Low	Bronze	3	SCM [MR]
A. amphibius	England, Kent	Ightham Fissures	Pleistocene	1	NHM (London) [MR]
A. amphibius	England, Kent	Ightham Fissures	Holocene	1	NHM (London) [MR]
A. amphibius	Wales, Gwent	Merlin's Cave	Younger Dryas	1	NHM (London) [MR]
A. amphibius	Wales, Gwent	Merlin's Cave	Holocene	1	NHM (London) [MR]
A. amphibius	France, Pyrennes	Poeymaü	Younger Dryas	1	MNP (France) [MR]
A. amphibius	France, Tarn-et-Garonne	Courbet	Pleistocene	1	NHM (London) [MR]
A. amphibius	Germany, Harz	Fuchsloch im Krockstein	Younger Dryas	3	NHM (Berlin) [MR]
A. amphibius	Germany, Malchin	Pisede bei Malchin	Holocene	3	NHM (Berlin) [MR]
A. amphibius	Hungary, Szergerd	Bélmegyer	Holocene	1	HIG [MR]
A. amphibius	Hungary, Budapest	Pilisszánto	Pleistocene	1	HIG [MR]
A. amphibius	Hungary, Bükk	Balla Cave	Pleistocene	1	HIG [MR]
A. amphibius	Italy, Veneto	Grotta del Broion	Pleistocene	3	UF (Italy) [MR]
A. amphibius	Slovakia	Dzeravá skala Cave	Pleistocene	1	HIG [MR]
A. amphibius	Ukraine		Recent	2	MIG [MR]
A. amphibius	Romania		Recent	1	IS (Romania) [MR]
A. amphibius	Belgium		Recent	2	NHM (London)
A. amphibius	England, Southeast		Recent	2	NHM (London)
A. amphibius	England, Mid		Recent	1	NHM (London)
A. amphibius	England, West		Recent	2	NHM (London)
A. amphibius	England, East Anglia		Recent	2	NHM (London)
A. amphibius	England, North		Recent	3	NHM (London)
A. amphibius	Scotland		Recent	2	NHM (London)
A. amphibius	Estonia		Recent	2	NHM (London)
A. amphibius	Finland		Recent	1	NHM (London)
A. amphibius	Italy		Recent	2	NHM (London)
A. amphibius	Macedonia		Recent	2	NHM (London)
A. amphibius	Norway		Recent	1	NHM (London)
A. amphibius	Russia		Recent	3	NHM (London)
A. amphibius	Sweden		Recent	2	NHM (London)
A. amphibius	Switzerland		Recent	1	NHM (London)
A. scherman	France		Recent	2	NHM (London)
A. scherman	Slovenia		Recent	1	NHM (London)
A. sapidus	Portugal		Recent	1	NHM (London)
A. sapidus	France		Recent	2	NHM (London)
			Total	**82**	

3.3.2 DNA extraction

All DNA extractions were conducted in a dedicated ancient DNA laboratory, physically separated from the post- PCR laboratory. Mandibles were ground into a fine powder and extracted using silica spin columns based on Yang *et al.* (1998) with the inclusion of 1M urea in the extraction buffer. MtDNA was amplified using overlapping fragments spanning 643 base pairs of the control region, tRNA-Phe gene and 12S ribosomal RNA regions. Six primer pairs were designed specifically for this study (*Appendix I*), each pair amplifying short (150 – 200 base pair) overlapping fragments. For all ancient DNA samples primer pairs were employed in 6 separate reactions, but for the amplification of modern material primer pairs were combined, thus requiring only 3 reactions per sample. PCR reactions were performed using a final concentration of 1 x PCR buffer, 0.2μM of each primer, 250μM dNTPs, 2mM $MgSO_4$, 1mg/ml BSA, 1 Unit Platinum Taq DNA polymerase high fidelity, purified water, and 2μl of DNA extract in a 25μl mix. PCR conditions were 5 min at 95°C, followed by 55 cycles of 1 min at 92°C, 1 min at between 50°C and 52°C (dependent on primer pair specifications), 1 min at 68°C, and with a final extension of 5 min at 68°C.

Amplicons were purified using Exonuclease I and Shrimp Alkaline Phosphatase. Sequencing reactions were performed by Macrogen (Korea) using a high throughput genetic analysis sequencer, ABI3730XL. Sequencing chromatograms were assembled and analysed using Sequencher 4.0 analysis software (Gene Codes Corporation). Standard ancient DNA protocols (Gilbert *et al.*, 2005) were followed throughout these extraction procedures to prevent contamination, with repeated PCR amplification and sequencing of fragments to ensure DNA authenticity and the absence of miscoding lesions.

3.3.3 Phylogenetic analyses

DNA sequences obtained from this study were aligned using Geneious Pro v5.03 (Drummond *et al.*, 2010). To provide additional sequence data, 27 unique modern haplotypes from the Piertney *et al.* (2005) dataset were identified and included in the alignment.

Phylogenetic relationships were estimated using Maximum Likelihood (ML) and Bayesian analysis. The DNA substitution model selected with ModelTest3.7 (Posada, Crandall, 1998) under Akaike Information Criterion (AIC) was General Time Reversible (GTR) with proportion of invariable sites (I) set to 0.6802 and gamma distribution (G) shape parameter 0.8091. Maximum Likelihood trees were generated in PAUP* (Swofford, 2000) using a full heuristic search, based on branch swapping with tree-bisection-reconnection (TBR). Bayesian trees were constructed using MrBayes 3.1 (Ronquist, Huelsenbeck, 2003) implementing nucleotide substitution model GTR, four chains (three heated one cold) were run for one million generations with a burn-in period of 1000 trees. Nodal support was determined using step-wise ML bootstrap analysis with 1000 replicates in PAUP* and by approximate posterior probabilities performed in MrBayes. Southern water vole (*A. sapidus*) (sample 208) from Portugal was employed as the outgroup in all analyses.

Sequence data were partitioned into haplogroups and Southern water vole species to establish sequence divergence between haplogroups and the Southern water vole. These were calculated in Arlequin Ver. 3.11 (Excoffier *et al.*, 2005), using pairwise estimates of corrected average population sequence divergence.

3.3.4 Radiocarbon dating

As part of the AHOBII project a subset of the water vole mandibles extracted in this study were also sent for accelerator mass spectrometry (AMS) dating at the Oxford radiocarbon accelerator unit. The small volume of sample material available limited the number of samples where sufficient volume remained to enable AMS dating. Dates were received as uncalibrated radiocarbon years BP (Before Present AD 1950), calibrated calendar ages were generated using Oxcal v4.1 (Ramsey, 2009) with IntCal09 calibration curve (Reimer *et al.*, 2009).

3.4 Results

3.4.1 DNA sequence data

From the 82 samples sourced in this study 70 successfully amplified water vole mtDNA. Three of these samples generated insufficient coverage, < 200 base pairs and were therefore excluded from analyses. Of the remaining 67 samples, 62 amplified the entire 643 base pair region of interest; a further 5 successfully amplified all but one primer pair (*Table 3.2*). To test whether the un-amplified regions contained informative data, phylogenies were generated using both the entire region of interest and with the un-amplified regions omitted. Trees produced were synonymous; the 5 partially amplified samples were therefore included in all further analyses.

3.4.2 Phylogenetic analyses

Maximum Likelihood and Bayesian analyses generated congruent phylogenetic trees, the Bayesian tree only is shown, with both the Bayesian approximated posterior probabilities and bootstrap values indicated (*Figure 3.3*). The phylogeny supports three clades of water voles, exhibiting division between samples from the Pleistocene and older Holocene samples. To clarify the geographical and temporal significance of these findings *Figures 3.4 – 3.6* illustrate the geographical location and age of samples, for each of the three clades in isolation.

Table 3.2 Amplification results and radiocarbon dates

My Identifier	Species	Location	Site	Approximate Age	Sucessfully Amplified	OxA Identifier	AMS 14C Date	Calibrated Date
117	A. amphibius	Belgium, Ardennes	Trou Al'Wesse	Holocene	Yes			
116	A. amphibius	Belgium, Ardennes	Trou Al'Wesse	Pleistocene	Yes			
118	A. amphibius	Belgium, Ardennes	Trou Al'Wesse	Pleistocene	Yes			
119	A. amphibius	Belgium, Ardennes	Trou Al'Wesse	Pleistocene	No			
120	A. amphibius	Belgium, Ardennes	Trou Al'Wesse	Pleistocene	No			
164	A. amphibius	Belgium, Ardennes	Trou du Frontal	Younger Dryas	No	OxA-21078	9,455 +/- 55	10,699
160	A. amphibius	Belgium, Namur	Marie-Jeanne Cave	Early Holocene	Yes			
161	A. amphibius	Belgium, Namur	Marie-Jeanne Cave	Pleistocene	575 bp (IA)			
163	A. amphibius	Belgium, Namur	Marie-Jeanne Cave	Pleistocene	No			
162	A. amphibius	Belgium, Namur	Marie-Jeanne Cave	Pleistocene	<200 bp (NIA)			
121	A. amphibius	England, Somerset	Bridged Pot	Younger Dryas	Yes			
122	A. amphibius	England, Somerset	Bridged Pot	Younger Dryas	Yes			
123	A. amphibius	England, Somerset	Bridged Pot	Younger Dryas	Yes			
124	A. amphibius	England, Somerset	Bridged Pot	Younger Dryas	Yes			
125	A. amphibius	England, Somerset	Bridged Pot	Younger Dryas	Yes			
156	A. amphibius	England, Somerset	Bridged Pot	Younger Dryas	Yes			
187	A. amphibius	England, Somerset	Gully Cave	Younger Dryas	Yes	OxA- 20249	12485 +/- 60	14,621
93	A. amphibius	England, Staffordshire	Ossom's Eyrie Cave	Roman	Yes			
101	A. amphibius	England, Staffordshire	Ossom's Eyrie Cave	Roman	Yes			
103	A. amphibius	England, Stafforshire	Thor's Fissure	Roman	Yes			
92	A. amphibius	England, Derbyshire	Pin Hole Lower Cave	Pleistocene	Yes			
97	A. amphibius	England, Derbyshire	Pin Hole Lower Cave	Pleistocene	Yes			
105	A. amphibius	England, Derbyshire	Pin Hole Lower Cave	Pleistocene	Yes			
104	A. amphibius	England, Derbyshire	Wigber Low	Bronze	Yes			
106	A. amphibius	England, Derbyshire	Wigber Low	Bronze	Yes			
107	A. amphibius	England, Derbyshire	Wigber Low	Bronze	Yes			
157	A. amphibius	England, Kent	Ightham Fissures	Pleistocene	No	OxA-21075	23,100 +/- 170	27,955
155	A. amphibius	England, Kent	Ightham Fissures	Holocene	<200 bp (NIA)	OxA-21074	8,560 +/- 40	9,532
158	A. amphibius	Wales, Gwent	Merlin's Cave	Younger Dryas	Yes	OxA-21076	10,295 +/- 45	12,081
159	A. amphibius	Wales, Gwent	Merlin's Cave	Holocene	Yes	OxA-21077	2,797 +/- 27	2,900
113	A. amphibius	France, Pyrennes	Poeymaü	Younger Dryas	<200 bp (NIA)	OxA-21079	10,065 +/- 45	11,611
154	A. amphibius	France, Tarn-et-Garonne	Courbet	Pleistocene	No			
99	A. amphibius	Germany, Harz	Fuchsloch im Krockstein	Younger Dryas	Yes	OxA-21118	11,110 +/-50	12,999
111	A. amphibius	Germany, Harz	Fuchsloch im Krockstein	Younger Dryas	575 bp (IA)	OxA-21111	10,035 +/- 50	11,538
110	A. amphibius	Germany, Harz	Fuchsloch im Krockstein	Younger Dryas	No	OxA-21082	11,005 +/- 50	12,874
95	A. amphibius	Germany, Malchin	Pisede bei Malchin	Holocene	Yes	OxA-2332	3,048 +/- 26	3,279
112	A. amphibius	Germany, Malchin	Pisede bei Malchin	Holocene	565 bp (IA)	OxA-2335	2,921 +/- 30	3,070
96	A. amphibius	Germany, Malchin	Pisede bei Malchin	Holocene	Yes	OxA-21080	2,546 +/- 26	2,687
100	A. amphibius	Hungary, Szergerd	Bélmegyer	Holocene	Yes			
90	A. amphibius	Hungary, Budapest	Pilisszánto	Pleistocene	Yes	OxA-21119	13,475 +/- 65	16,653
109	A. amphibius	Hungary, Bükk	Balla Cave	Pleistocene	No	OxA-21112	12,515 +/- 60	14,681
94	A. amphibius	Italy, Veneto	Grotta del Broion	Pleistocene	No			
98	A. amphibius	Italy, Veneto	Grotta del Broion	Pleistocene	No			
102	A. amphibius	Italy, Veneto	Grotta del Broion	Pleistocene	No			
91	A. amphibius	Slovakia	Dzerává skala Cave	Pleistocene	Yes	OxA-21081	22,410 +/- 150	27,137
114	A. amphibius	Ukraine		Recent	565 bp (IA)			
115	A. amphibius	Ukraine		Recent	Yes			
108	A. amphibius	Romania		Recent	Yes			
207	A. amphibius	Belgium		Recent	No			
212	A. amphibius	Belgium		Recent	Yes			
194	A. amphibius	England, Southeast		Recent	Yes			
198	A. amphibius	England, Southeast		Recent	Yes			
201	A. amphibius	England, East Anglia		Recent	Yes			
199	A. amphibius	England, Northern		Recent	Yes			
206	A. amphibius	England, Mid		Recent	Yes			
200	A. amphibius	England, North		Recent	Yes			
195	A. amphibius	England, North		Recent	Yes			
204	A. amphibius	England, West		Recent	Yes			
205	A. amphibius	England, West		Recent	Yes			
197	A. amphibius	England, East Anglia		Recent	Yes			
202	A. amphibius	Scotland		Recent	Yes			
196	A. amphibius	Scotland		Recent	Yes			
211	A. amphibius	Estonia		Recent	Yes			
226	A. amphibius	Estonia		Recent	Yes			
218	A. amphibius	Finland		Recent	Yes			
215	A. amphibius	Italy		Recent	Yes			
203	A. amphibius	Italy		Recent	Yes			
224	A. amphibius	Macedonia		Recent	Yes			
227	A. amphibius	Macedonia		Recent	Yes			
220	A. amphibius	Norway		Recent	Yes			
210	A. amphibius	Russia		Recent	Yes			
225	A. amphibius	Russia		Recent	575 bp (IA)			
223	A. amphibius	Russia		Recent	Yes			
214	A. amphibius	Sweden		Recent	Yes			
216	A. amphibius	Sweden		Recent	Yes			
219	A. amphibius	Switzerland		Recent	Yes			
213	A. scherman	France		Recent	Yes			
221	A. scherman	France		Recent	Yes			
222	A. scherman	Slovenia		Recent	Yes			
208	A. sapidus	Portugal		Recent	Yes			
217	A. sapidus	France		Recent	Yes			
209	A. sapidus	France		Recent	Yes			

Dates are recorded as years Before Present (BP), the calibrated date represents the median date calibrated in OxCal with 95% confidence interval. Sequence amplification: IA = included in analyses; NIA = not included in analyses; bp = number of base pairs, applied when amplification was less than the target 643 base pair region.

Figure 3.3 *Phylogeny for the water vole*
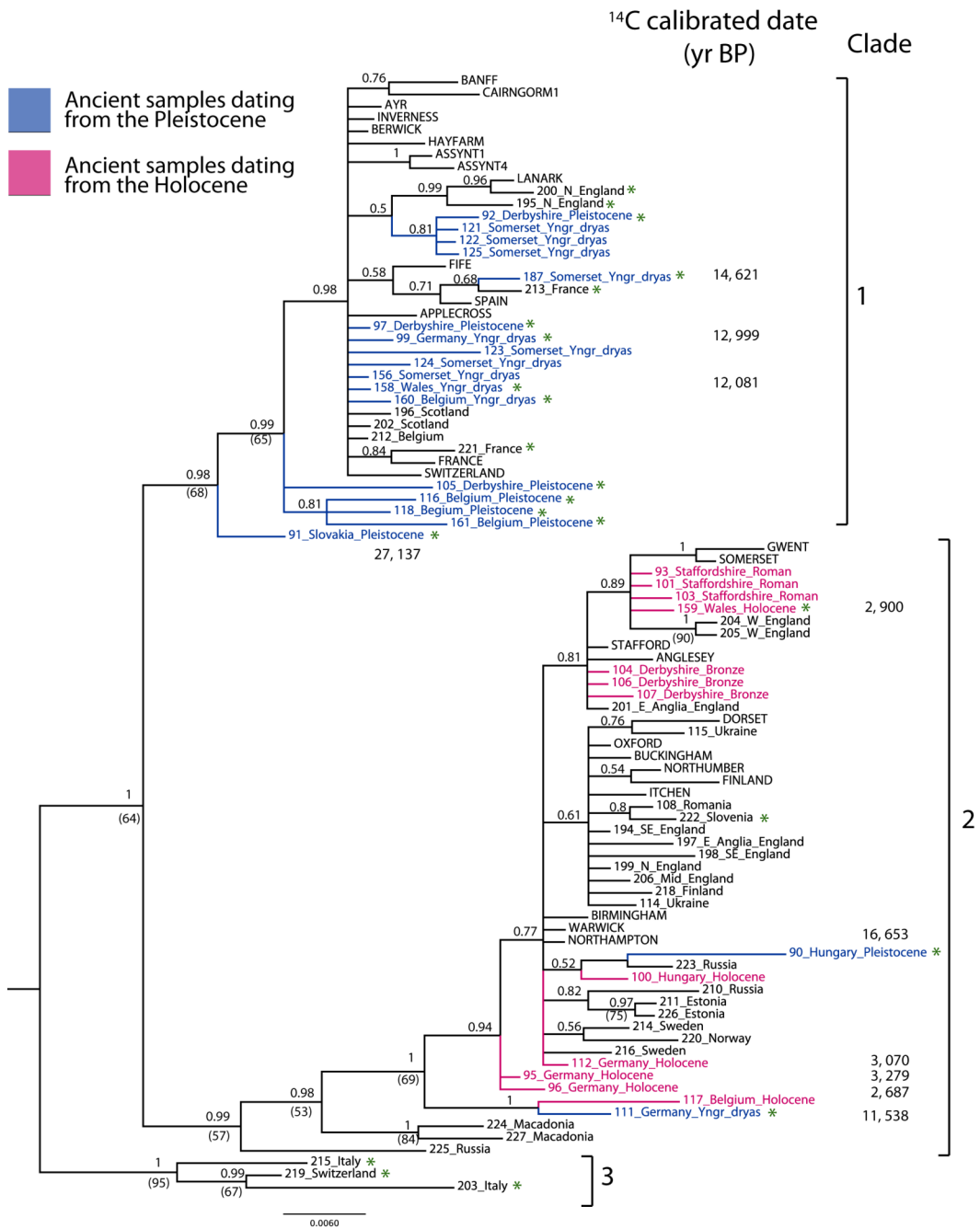
Bayesian tree constructed in MrBayes with GTR for 1000,000 generations. Nodal support is shown through approximate Bayesian probabilities above the line and bootstrap values in parentheses (only values above 50% shown). AMS dates are given as median calibrated yr BP. Southern water vole used as an outgroup (not shown). Nomenclature: Data taken from Piertney et. al (2005)are written in capitals, all other data are from this study, 1st number = my sample identifier, followed by location and time period, blue = Pleistocene, pink = Holocene, black = modern. Green star denotes that the sample is directly referred to in the text.

Figure 3.4 *Location of water vole samples from Clade 1*
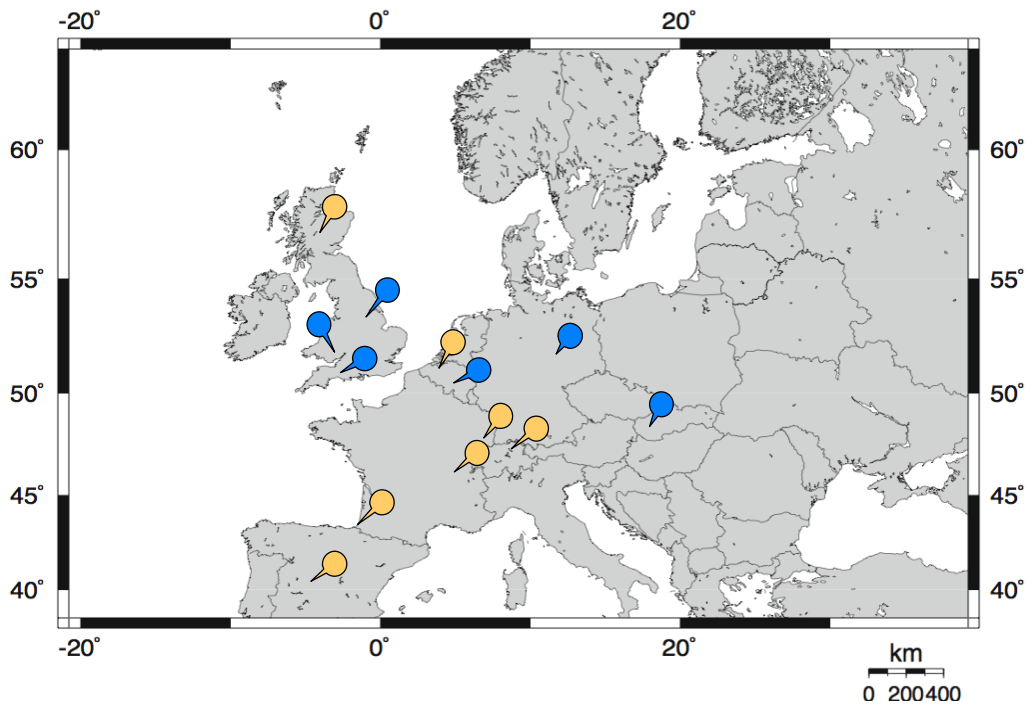
Samples from the Pleistocene are coloured blue and modern samples yellow

Figure 3.5 *Location of water vole samples from Clade 2*
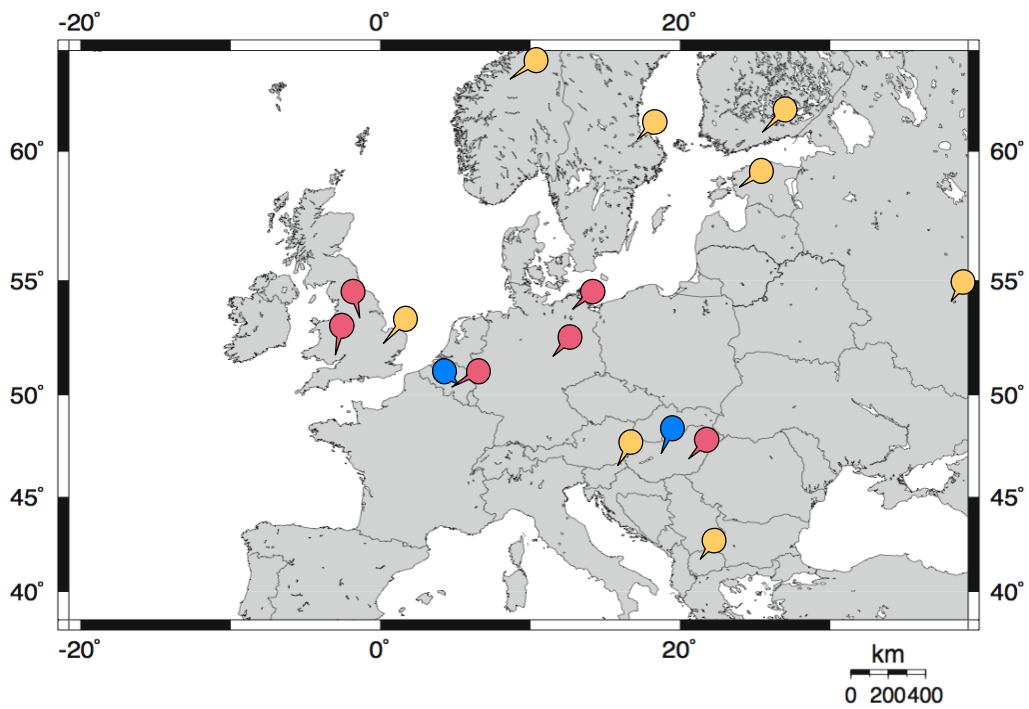
*Samples from the Pleistocene are coloured blue; Holocene samples pink and modern
samples yellow*

Figure 3.6 *Location of water vole samples from Clade 3*

Yellow colour denotes modern samples only
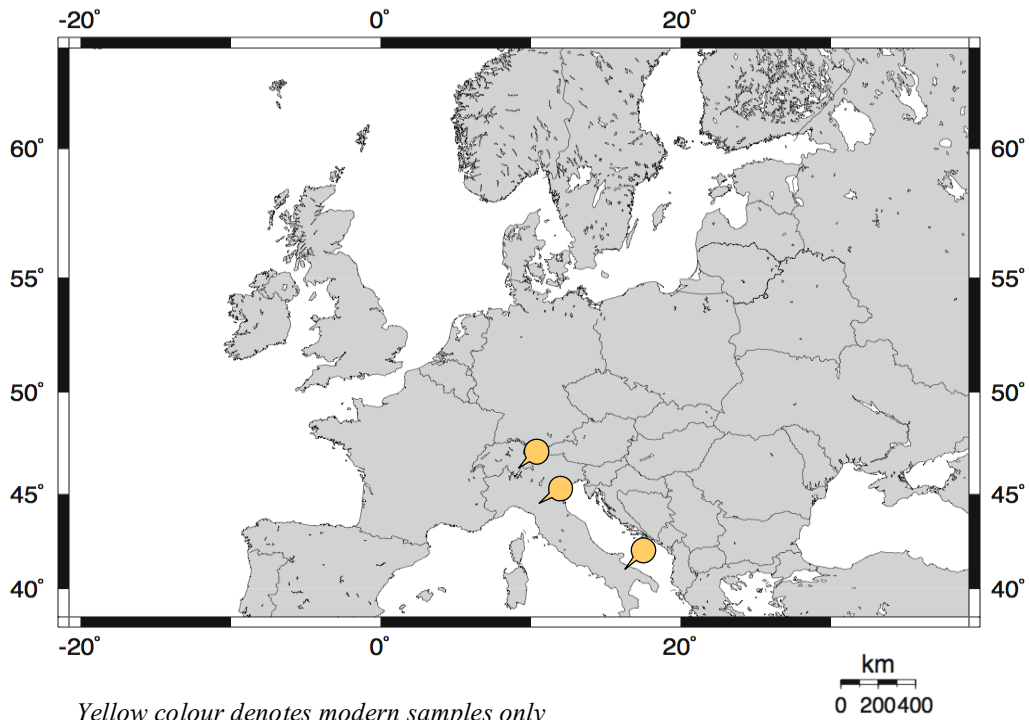
Further to clade identification, sequence data were partitioned to assess the percentage of sequence divergence between the haplogroups identified and the sister species, the Southern water vole (*Table 3.3*).

Table 3.3 *Population average pairwise estimates of sequence divergence with Kimura-2 Parameters between haplogroups and the Southern water vole (A. sapidus)*

		Clade			All
		1	2	3	*A.* amphibius
Clade	1				
	2	2.12			
	3	2.42	2.49		
All	*A. sapidus*	3.9	4.56	3.3	3.66

Data represent the percentage sequence difference between each clade identified population and the Southern water vole (A. sapidus).

3.5 Discussion

The main aim of this study was to investigate water vole colonisation of the UK, through the application of phylogenetic analysis. This incorporated original, modern samples from across the UK, to facilitate exploration of a major (molecular) division between modern water vole in England and those in Scotland (Piertney *et al.*, 2005). The phylogeny constructed (*Figure 3.3*) placed all modern Scottish samples as part of a single clade, identified here as clade 1 and most modern English samples in an additional clade, denoted clade 2. There are two exceptions to this trend, sample 195 (Read's Island) and 200 (Northumberland) both from English locations, but genetically associated within the Scottish haplogroup, clade 1. The location of the Northumberland sample is directly adjacent to the Scottish border, its close proximity likely accounts for its association with the 'Scottish' clade. Read's Island, however, provides more of an anomaly; an RSPB reserve situated in the Humber Estuary, its location although in the North of England is considerably to the south of the Scottish border. I have since ascertained that this is not a unique occurrence, a limited number of similar anomalies have been discerned, primarily samples close to the Scottish border, (S. Piertney, unpublished). However, the overall trend of a major division between water vole in the north and the south of the UK persists, as evidenced by the strong statistical support for the nodes that define lineage separation into haplogroup clusters.

Confirmation of the genetic division observed by Piertney *et al.* (2005) can be achieved through modern sampling efforts, but to determine a more comprehensive explanation of colonisation events, ancient Pleistocene and Holocene water vole samples were included in the analysis. These are denoted in the phylogeny (*Figure 3.3*) by geographic location and coloured blue (Pleistocene) and pink (Holocene). The phylogenetic placement of these samples is illuminating; Pleistocene samples from England share the same haplogroup as those currently restricted to Scotland, clade 1. Only Holocene samples, those post dating the last cold phase, the Younger Dryas (ca. 12.8 – 11.5 Kyr BP) from England cluster within the modern English water vole clade (clade 2). That Pleistocene samples from England cluster with modern Scottish samples implies

that members of this genetic haplogroup were the initial colonisers of the UK, and found throughout England during the Pleistocene. After the end of the Younger Dryas this group were displaced by a second wave of colonisers, the genetic signature apparent in clade 2, these were present in England during the Holocene through to the current day. The second wave of colonisers replaced the first in England, either driving or limiting the initial colonisers to Scotland. The phylogenetic pattern provides a clear indication that water vole colonised the UK on (at least) two separate occasions, thus colonisation events were temporally as opposed to geographically separated. This reconstruction of events also presents a potential explanation for anomalous samples such as Read's Island (reported here), as representatives of relict populations from the initial colonisers, isolated remnants of the original colonisers before they were displaced to Scotland.

Timing of the second colonisation event can be inferred from the direct dating of samples used in this study (*Table 3.2* and *Figure 3.3*). Three samples from the UK were AMS dated, 2 of which were dated to before the termination of the Younger Dryas (all dates are median calibrated dates); sample 187 was dated to 14, 621 BP and sample 158, returned a date of 12, 081 BP, both are from haplogroup 1. The third sample, 159, dated to the Holocene, 2, 900 BP and is associated with the second wave of colonisers, clade 2, thus the second colonisation event occurred between 12, 081 and 2, 900 years BP. The second wave of colonisers would have presumably required land access to the UK from Europe. The last land bridge between England and Europe is estimated to have been ca. 8000 BP (Weninger *et al.*, 2008) after which 'Doggerland' (the connecting land area) submerged with the formation of the English Channel. Colonisation must therefore have occurred prior to this date. Two colonisation events, with the second phase occurring post the Younger Dryas have previously been hypothesised for several small mammal species in the UK; the common shrew (*Sorex araneus*) (Searle, Wilkinson, 1987); the bank vole (*Myodes glareolus*), field vole (*Microtus agrestis*) and pygmy shrew (*Sorex minutus*) (Searle *et al.*, 2009). My research, however, represents the first to utilise directly dated ancient DNA samples to definitively illustrate that two temporally separated colonisation events occurred in the UK.

The incorporation of ancient DNA has thus provided clarity as to the timing and order of water vole colonisation events in the UK. Unfortunately, though, it does not establish why the second wave of colonisers replaced the first. Timing of this event must have occurred between ca. 12 – 3 Kyr BP according to the dated phylogeny recovered from this study, but presumably before the loss of the land bridge with Europe, restricting the time period to between 12 and 8 Kyr BP. This was a period of oscillating climate, spanning the last cold phase, the Younger Dryas and the post-warming phase that followed.

Population displacements are most commonly associated with one population out competing another, through some advantage either environmentally or physiologically driven. Water voles from Scotland are generally considered smaller and typically darker in colour than those from England (Woodroffe *et al.*, 2008), but morphological studies have shown this to be a continuum across the UK and do not consider the variation to support species or subspecies level differences (Corbet *et al.*, 1970; Montgomery, 1975). However, in the early 1900's water vole in Scotland were considered a subspecies, *A. terrestris reta* (Miller, 1910) based on their darker melanic pelage. Further, a study by Turk (1964) found Bronze Age water vole skulls from Derbyshire, England to be more akin to the Scottish *reta* subspecies than to the English subspecies, *A. t amphibius*. Turk postulated that either the early Bronze Age population differentiated into two subspecies or that the *reta* population was once common in England, but replaced by a second population (*amphibius*) post the Bronze Age. This led Van den Brink (1967) to allocate two species of UK water vole, suggesting that the northerly species had been driven back to the Scottish Highlands by the species from the south. Both authors were derided; Montgomery (1975) asserted that the insufficient sample size of Turk (1964), had led to the erroneous identification of two species, and that only one species of water vole had been present in Central England during the past 12,000 years. However, in light of the findings from this study, Turk and Van den Brink appear to have been correct with regards to a displacement event due to a second colonisation, even if the proposed timings were inaccurate.

Despite a lack of morphological differences the evidence collated here illustrates that a second wave of colonisers did displace the first in England. As there are no reports of discernable ecological or physiological differences between the two, yet population replacement occurred, an alternative supposition is that the colder climate of the Younger Dryas heralded a severe reduction in water vole numbers. As climate warmed, the second colonisers arrived to a region virtually devoid of water voles, resulting in complete genetic replacement in the South. Meanwhile in the North, the more heterogeneous landscape may have afforded increased pockets for survival, and as climate warmed these small populations recovered and repopulated. The additional time, due to increased distance from the point of entry of the second colonisers (across 'Doggerland' in the South), may also have aided population recovery, thus sufficient numbers were in place to avoid genetic replacement. Alternatively it is possible that the first colonisers were more disposed to cold tolerance, ensuring survival in the North, but to date there is no physical evidence to support this.

When the broader geographical outlook is considered, the Pleistocene/Holocene division can be observed across Europe. Pleistocene samples from Slovakia, Belgium and Germany are found in haplogroup 1, where as Holocene samples, Belgium and Germany are within haplogroup 2. This suggests post Younger Dryas population replacement may have occurred in these regions. Belgium is an area of particular note, as the modern sample reverts to a clade one haplotype. This could indicate that both haplotypes remain in the region, highlighting the possibility of a Belgian suture zone. That only one modern Belgian sample successfully yielded DNA provides insufficient support to test this line of investigation, but with greater sampling effort in the region, this question could be resolved.

Germany also warrants additional discussion as dated Pleistocene samples from the same site, (Fuchsloch im Krockstein) can be found in both clade 1 and clade 2. Sample 99, a clade 1 haplotype, dates just prior to the Younger Dryas, while sample 111, a clade 2 haplotype, has a median calibrated date of 11, 538 BP, the very cusp of the Younger Dryas/Holocene boundary. It is therefore possible that the time between these two samples, essentially the Younger Dryas, represents a

more accurate estimate of the timing of the second colonisation event. Alternatively, Germany may also form part of a suture zone with both haplotypes present. As with Belgium I believe this could be resolved with further sampling efforts, unfortunately beyond the scope of this study.

Although not exhaustive, the additional modern samples from throughout Europe, included in this analysis, illustrate a lineage division between the east and the west. This is congruent with previous studies, used as evidence for the southern refugia model (Taberlet *et al.*, 1998). However, the inclusion of ancient DNA within this analysis affords a greater depth of knowledge. According to the Hewitt model, Central and Northern Europe were recolonised by a limited number of founder individuals from the South, thus limiting the gene pool, with current haplogroups reflecting the genetic signature of the refugium in which they survived. This implies that genetic signature from non-refugium, (Central and Northern Europe) prior to the LGM would be lost due to extinction, providing no contribution or association with current populations. Under these conditions, pre-LGM ancient DNA samples from non-refugium locations included in a modern phylogeny would be expected to form a separate lineage, disassociated from modern and southern refugia haplotypes.

The phylogenetic structure collated here, however, provides evidence to the contrary, with pre-LGM samples from across Europe associating within modern haplogroup clades. Sample 161 (Belgium, Marie-Jeanne cave) originated from a deposit with a minimum age ca. 40 Kyr BP; samples 116 and 118 (Belgium, Trou Al'Wesse) and samples 92, 97 and 105 (UK, Derbyshire, Pin Hole) are associated with pre LGM layers; and sample 91 (Slovakia) has been directly dated to 27 Kyr BP, again prior to the LGM. All of these samples fall within clade 1, and are more closely related to samples in Scotland today than to Holocene samples from their own geographical location (clade 2). This illustrates that current populations are not exclusively derived from refugia populations that recolonised the North via founder events. Instead, this study highlights genetic contribution of pre-LGM non-refugial populations, indicating that a strict refugial model of postglacial recolonisation originating solely from southern peninsulas is likely to be an oversimplification of events. It also confers the importance of examining a

spectrum of species, including small mammals, as water vole appear to exhibit an unusual pattern of genetic diversity, in that the transition from the Late Pleistocene into the Holocene did not result in a major decline in genetic diversity. This runs contrary to most other species (Hofreiter, Barnes, 2010), including collared lemmings (*Dicrostonyx torquatus*) (chapter 2 & Prost *et al.*, 2010).

A final interpretation of the dataset aimed to ascertain whether current water vole taxonomy could be validated through a molecular approach. The results indicated clear confirmation of the species level status of the Southern water vole (*A. sapidus*), through the monophyly of samples and the high percentage of sequence divergence between Southern and Northern water vole, 3.66% (*Table 3.3*). However, based on mtDNA I found no basis for determining the montane water vole (*A. scherman*) to species level. Three modern samples (213, 221, 222) classified montane water vole by the Natural History Museum (London) failed to form a cohesive monophyletic association, further, they were designated to different haplogroups, samples 213 and 221 (France) were clade 1 haplotypes, while the sample from Slovenia (222) was a clade 2 haplotype.

A far clearer lineage separation was apparent in samples from Italy (203 and 215) and Switzerland (located south of the Alps), sample 219. Together, these modern samples form a monophyletic clade, clade 3, with relatively high sequence divergence between this clade and either clade 1 (2.42%) or clade 2 (2.49%), with divergence not considerably lower than the species level difference between Northern and Southern water vole (3.66%), (*Table 3.3*). From a purely molecular perspective the close association of this haplogroup and sequence divergence from other groups provide a more convincing argument of speciation than that obtained from the montane water vole, although it is important to stress that this is exclusively based on control region mtDNA, and that the inclusion of nuclear DNA could provide contrary evidence of species level divergence in the montane water vole. I therefore suggest that the species status of the montane water vole warrants further investigation and would benefit from molecular studies, including nuclear DNA, to specifically address the findings identified here.

In summary, this study highlights the benefits and increased depth of knowledge that can be obtained from incorporating an ancient DNA approach to evolutionary conundrums. Modern data was sufficient to identify molecular distinction between English and Scottish water voles, but unable to detect details of events prior to this outcome. This study was able to reveal a more comprehensive explanation of lineage separation observed in UK water vole today, through phylogenetic analysis of ancient and modern DNA. It resolved the order and timing of UK colonisation events, defining two temporally distinct events, where a second lineage of water vole replaced the first in England ca. 12 – 8 Kyr BP, leaving the first UK colonisers restricted to Scotland. This study has further contributed to knowledge on European postglacial recolonisation routes and the growing body of evidence that a strict southern refugial model need not be the most appropriate explanation for all species. Finally, although I see little merit in continually revising water vole nomenclature, I note that, within the context of an mtDNA investigation, there is a distinct lack of coherent structure that can be attributed to samples identified to the species level *A. scherman* (montane water vole).

3.6 References

Ashby KR, Harling J, Whiles D (1969) Observations on the distribution and activity of Water voles (*Arvicola amphibius amphibious*) in Old Durham Beck, Durham. *Journal of Zoology* **158**, 209-214.

Bilton DT, Mirol PM, Mascheretti S*, et al.* (1998) Mediterranean Europe as an area of endemism for small mammals rather than a source for northwards postglacial colonization. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **265**, 1219-1226.

Blasius JH (1857) Fauna der Wirbelthiere Deutschlands und der angrenzenden Länder von Mitteleuropa: Naturgeschichte der Säugethiere Deutschlands und der angrenzenden Länder von Mitteleuropa, pp. 330 -397.

Brito PH (2005) The influence of Pleistocene glacial refugia on tawny owl genetic diversity and phylogeography in western Europe. *Molecular Ecology* **14**, 3077-3094.

Corbet GB, Cummins J, Hedges SR, Krzanowski W (1970) The taxonomic status of British Water voles, genus *Arvicola*. *Journal of Zoology* **161**, 301-316.

Dalén L, Nyström V, Valdiosera C*, et al.* (2007) Ancient DNA reveals lack of postglacial habitat tracking in the arctic fox. *Proceedings of the National Academy of Sciences* **104**, 6726-6729.

Drummond AJ, Ashton B, Buxton S*, et al.* (2010) Geneious.

Eldredge N (1989) *Macroevolutionary Dynamics: Species, Niches and Adaptive Peaks* McGraw-Hill, New York.

Eldredge N (1995) *Reinventing Darwin: The Great Evolutionary Debate* Weidenfeld and Nicolson.

Ellerman JR, Morrison-Scott TCS (1951) *Checklist of Palaearctic and Indian mammals 1758 to 1946* British Museum of Natural History (London).

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): An integrated software package for population genetics data analysis. Libertas Academica.

Gilbert MTP, Bandelt H-Jr, Hofreiter M, Barnes I (2005) Assessing ancient DNA studies. *Trends in Ecology & Evolution* **20**, 541-544.

Haffer J (1969) Speciation in Amazonian Forest Birds. *Science* **165**, 131-137.

Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* **58**, 247-276.

Hewitt GM (1999) Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society* **68**, 87-112.

Hewitt GM (2000) The genetic legacy of the Quaternary ice ages. *Nature* **405**, 907-913.

Hewitt GM (2001) Speciation, hybrid zones and phylogeography — or seeing genes in space and time. *Molecular Ecology* **10**, 537-549.

Hinton MAC (1926) *Monograph of the voles and lemmings (Microtinae) living and extinct* British Museum of natural History (London).

Hofreiter M, Barnes I (2010) Diversity lost: are all Holarctic large mammal species just relict populations? *BMC Biology* **8**, 46.

Jefferies DJ, Morris PA, Mulleneux JE (1989) An enquiry into the changing status of the Water Vole *Arvicola terrestris* in Britain. *Mammal Review* **19**, 111-131.

Kotlík P, Deffontaine V, Mascheretti S, *et al.* (2006) A northern glacial refugium for bank voles (Clethrionomys glareolus). *Proceedings of the National Academy of Sciences of the United States of America* **103**, 14860-14864.

Lawton JH, Gordon LW (1991) Habitat and the Distribution of Water Voles: Why are there Gaps in a Species' Range? *Journal of Animal Ecology* **60**, 79-91.

Linnaeus C (1758) *Systema naturae*, 10th edn.

Lunt DH, Ibrahim KM, Hewitt GM (1998) mtDNA phylogeography and postglacial patterns of subdivision in the meadow grasshopper Chorthippus parallelus. *Heredity* **80**, 633-641.

Martínez-Solano I, Teixeira J, Buckley D, García-París M (2006) Mitochondrial DNA phylogeography of *Lissotriton boscai* (Caudata, Salamandridae): evidence for old, multiple refugia in an Iberian endemic. *Molecular Ecology* **15**, 3375-3388.

Miller GS (1910) Brief synopsis of the Water rats of Europe. *Proceedings of the Biological Society of Washington* **23**, 19 - 22.

Miller GS (1912) *Catalogue of the mammals of western Europe (Europe exclusive of Russia) in the collection of the British Museum* British Museum of Natural History (London).

Montgomery WI (1975) On the relationship between sub-fossil and recent British Water voles. *Mammal Review* **5**, 23-29.

Musser GG, Carleton MC (2005) Superfamily Muroidea. In: *Mammal Species of the World. A Taxonomic and Geographic Reference* (eds. Wilson DE, Reeder DM), pp. 963 - 966. Johns Hopkins University Press, Baltimore.

Perry JS (1943) Reproduction in the Water-Vole, Arvicola amphibius Linn. *Proceedings of the Zoological Society of London* **A112**, 118-130.

Piertney SB, Stewart WA, Lambin X*, et al.* (2005) Phylogeographic structure and postglacial evolutionary history of water voles (Arvicola terrestris) in the United Kingdom. *Molecular Ecology* **14**, 1435-1444.

Posada D, Crandall K (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817-818.

Prost S, Smirnov N, Fedorov VB*, et al.* (2010) Influence of Climate Warming on Arctic Mammals? New Insights from Ancient DNA Studies of the Collared Lemming Dicrostonyx torquatus. *PLoS one* **5**, e10447.

Provan J, Bennett KD (2008) Phylogeographic insights into cryptic glacial refugia. *Trends in Ecology & Evolution* **23**, 564-571.

Ramsey B, C. (2009) Bayesian analysis of radiocarbon dates. *Radiocarbon* **51**, 337-360.

Reimer PJ, Baillie MGL, Bard E*, et al.* (2009) INTCAL09 AND MARINE09 radiocarbon age calibration curves, 0-50,000 years cal BP. *Radiocarbon* **51**, 1111-1150.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572-1574.

Searle JB, Kotlík P, Rambau RV*, et al.* (2009) The Celtic fringe of Britain: insights from small mammal phylogeography. *Proceedings of the Royal Society B: Biological Sciences* **276**, 4287-4294.

Searle JB, Wilkinson PJ (1987) Karyotypic variation in the common shrew (Sorex araneus) in Britain - a "Celtic Fringe". *Heredity* **59**, 345-351.

Stewart JR, Lister AM (2001) Cryptic northern refugia and the origins of thee modern biota. *Trends in Ecology and Evolution* **16**, 608-613.

Stewart JR, Lister AM, Barnes I, Dalén L (2010) Refugia revisited: individualistic responses of species in space and time. *Proceedings of the Royal Society B: Biological Sciences* **277**, 661-671.

Svendsen JI, Alexanderson H, Astakhov VI*, et al.* (2004) Late Quaternary ice sheet history of northern Eurasia. *Quaternary Science Reviews* **23**, 1229-1271.

Swofford DL (2000) Phylogenetic analysis using parsimony (* and other methods),. Sinaur Associates, Sunderland, Massachusetts.

Taberlet P, Fumagalli L, Wust-Saucy A-G, Jean-François. C (1998) Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology* **7**, 453-464.

Turk FA (1964) On some Bronze Age remains of the water-rat (*Arvicola terrestris amphibius* L). *Proceedings of the Zoological Society of London* **143**, 345-350.

Valdiosera CE, García N, Anderung C*, et al.* (2007) Staying out in the cold: glacial refugia and mitochondrial DNA phylogeography in ancient European brown bears. *Molecular Ecology* **16**, 5140-5148.

Valdiosera CE, García-Garitagoitia JL, Garcia N*, et al.* (2008) Surprising migration and population size dynamics in ancient Iberian brown bears (Ursus arctos). *Proceedings of the National Academy of Sciences* **105**, 5123-5128.

Van den Brink FH (1967) A Field Guide to the Mammals of Britain and Europe, pp. 98 - 100. Collins, London.

Ventura J, Casado-Cruz M (2010) Post-weaning ontogeny of the mandible in fossorial water voles: ecological and evolutionary implications. *Acta Zoologica* no. doi: 10.1111/j.1463-6395.2010.00449.x.

Weninger B, Schulting R, Bradtmöller M*, et al.* (2008) The catastrophic final flooding of Doggerland by the Storegga Slide tsunami. *Documenta Praehistorica* **35**, 1-24.

Willis KJ (2000) The Full-Glacial Forests of Central and Southeastern Europe. *Quaternary Research* **53**, 203-213.

Wójcik JM, Kawaałko A, Marková S, Searle JB, Kotlἰk P (2010) Phylogeographic signatures of northward post-glacial colonization from high-latitude refugia: a case study of bank voles using museum specimens. *Journal of Zoology* **281**, 249-262.

Woodroffe GL, Lambin X, Strachan R (2008) Genus Arvicola. In: *Rodents Order Rodentia* (compiled by Gurnell, J. Hare, EJ) In: *Mammals of the British*

Isles: Handbook, 4th Edition (eds. Harris S, Yalden DW), pp. 110 - 117. The Mammal Society, Southampton, England.

Yang DY, Eng B, Waye JS, Dudar JC, Saunders SR (1998) Improved DNA extraction from ancient bones using silica-based spin columns. *American Journal of Physical Anthropology* **105**, 539-543.

Chapter 4: Population history of the Hispaniolan hutia

4.1 Abstract

Hispaniola is a geotectonically complex island consisting of two palaeo-islands that docked ca. 10 Myr, with a further geological boundary subdividing the southern palaeo-island into eastern and western regions. All three regions have been isolated by marine barriers during the late Cenozoic, and possess biogeographically distinct terrestrial biotas. However, there is currently little evidence to indicate whether Hispaniolan mammals show distributional patterns reflecting this geotectonic history, as the island's recent land mammal fauna is now almost entirely extinct. I obtained samples of Hispaniolan hutia (*Plagiodontia aedium*), one of two surviving Hispaniolan land mammal species, from fieldwork and historical museum collections from seven localities distributed across all three of the island's biogeographic regions. Phylogenetic analysis using mitochondrial DNA (cytochrome *b*) reveals a pattern of historical allopatric lineage divergence in this species, with three distinct hutia lineages biogeographically consistent with the island's geotectonic history. Coalescent modelling analyses indicate near-complete genetic isolation of each hutia lineage. However, this spatial congruence is not matched by temporal congruence to the unification of the palaeo-islands ca. 10 Myr, as mutation rates indicate that divergence between northern and southern hutia lineages occurred significantly later.

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4.3 Introduction

Non-volant land mammals have rarely colonised non-continental (oceanic-type) islands, in contrast to many other vertebrate groups. One of the few diverse evolutionary radiations of land mammals on oceanic-type islands took place in the Caribbean region, which consists of a complex series of geotectonic units not associated with the continental shelf regions of North or South America (Iturralde-Vinent, MacPhee 1999; Graham 2003). The terrestrial Holocene insular Caribbean mammal fauna formerly consisted of approximately 120 endemic species of sloths, eulipotyphlan insectivores, primates and rodents (MacPhee 2009; Turvey 2009). However, over 100 land mammal species or island populations have become extinct following mid-Holocene Amerindian colonisation and subsequent historical-era European settlement of the region (MacPhee, Flemming 1999; MacPhee 2009; Turvey 2009).

The different mammalian lineages that made up the pre-human land mammal fauna of the insular Caribbean probably originated through ancient vicariance, over-water dispersal, and possibly also dispersal over a geologically transient landspan (Iturralde-Vinent, MacPhee 1999; Hedges 2001, 2006; Roca *et al*. 2004) (*Chapter 1, section 1.6, Caribbean*). However, understanding the evolutionary processes that drove subsequent diversification and speciation in this regional fauna have been confused by limited preservation of Tertiary fossil deposits, ongoing taxonomic problems, and rapid degradation of ancient DNA in humid subtropical environments (Turvey 2009). In particular, it is unclear whether intra-island speciation has been a significant factor in generating observed patterns of species diversity in the region's Holocene mammal fauna. Several closely related species groups (e.g. capromyine, isolobodontine and plagiodontine hutias, heteropsomyine spiny rats, nesophontid island-shrews) apparently occurred sympatrically in the Greater Antilles during the Late Quaternary, but species delimitations and phylogenetic interrelationships in these radiations are confused

(Díaz-Franco 2001; Condis Fernández *et al*. 2005). Although phylogeographic investigation of surviving Caribbean mammal taxa has the potential to assess whether sister pairs of insular land mammal lineages arose sympatrically or through periods of inter-island or intra-island allopatry, studies addressing this significant evolutionary question have not yet been attempted. However, phylogeographic studies of other endemic species radiations have been conducted for several terrestrial vertebrate groups from Hispaniola, a large, topographically heterogeneous island which contains multiple endemic species pairs of many vertebrate taxa, and which is also one of the few Caribbean islands to retain native land mammals.

Hispaniola, divided politically into the Dominican Republic and Haiti, has a complex Cenozoic geotectonic history (*Figure 4.1*). The island consists of independent northern and southern palaeo-islands which docked by the late Miocene ca. 10Myr (Mann *et al*. 1991; Iturralde-Vinent, MacPhee 1999), but which remained separated by a prominent depression, the Neiba Valley or Cul-de-Sac/Enriquillo Graben. This was permanently or periodically inundated to form a narrow seaway until the Late Pleistocene, providing a potential barrier to gene flow (Maurrasse *et al*. 1982; Graham 2003). The southern palaeo-island, a 350-kilometre long oceanic plateau terrane, experienced progressive uplift during the Neogene and formed a largely emergent peninsula by the Pliocene subdivided into two major physiographic provinces, the Massif de la Hotte in the west and the Massif de la Selle and Sierra de Baoruco in the east. These provinces are separated by a structural low bisecting the peninsula, the Jacmel-Fauché depression, which was also inundated by a sea channel during some or all of the Pliocene and Pleistocene (Maurrasse *et al*. 1982).

Figure 4.1 *Hispaniola: geotectonic units and contemporary location and boundaries*
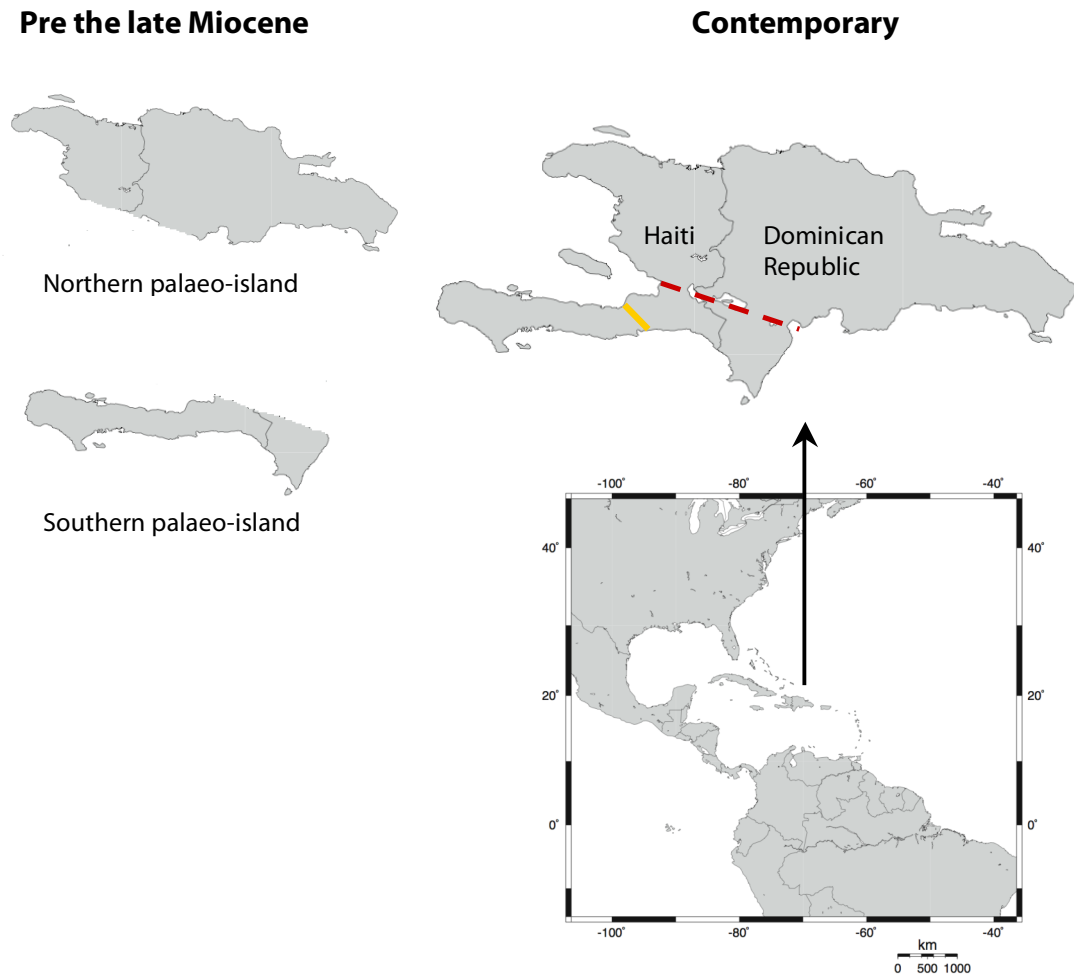
Schematic representation of Hispaniola pre the late Miocene as two separate units and post the late Miocene - current position - within the Caribbean. The broken red line represents the Neiba Valley (palaeo-island division) the solid yellow line represents the Jacmel-Fauché depression (Bond's Line)

All three of these regions are biogeographically distinct and are characterized by substantial levels of endemism in birds, reptiles, amphibians, invertebrates and plants. Distinct north and south Hispaniolan island biotas have been recognised for over 70 years, and the Jacmel-Fauché depression is sometimes referred to as a biogeographic boundary called 'Bond's Line'; these congruent biogeographic patterns of intra-island endemism are interpreted as reflecting historical allopatry (Mertens 1939; Williams 1961; Schwartz 1980; Hedges 1999; Latta *et al*. 2006).

This model of ancient allopatric differentiation of components of Hispaniola's terrestrial fauna has been supported by genetic analysis of several Hispaniolan terrestrial vertebrates. Townsend *et al*. (2007) found that chat-tangers (*Calyptophilus*) represent two lineages that correspond spatially and temporally to the Miocene division of Hispaniola into two palaeo-islands, and Gifford *et al*. (2004) and Gifford, Larson (2008) demonstrated that allopatric lineage divergence of northern and southern populations of the teiid lizard (*Ameiva chrysolaema*) was associated with Pliocene marine inundation of the Neiba Valley. Losos, Schluter (2000) also proposed that anole (*Anolis)* superspecies complexes on Hispaniola evolved through allopatric lineage divergence associated with its historical subdivision into several closely situated islands, and some evidence for a north-south palaeo-island division between sister taxa in the Hispaniolan *Anolis cybotes* complex was shown by Glor *et al*. (2003). Similar *in situ* spatial processes of historical terrane accretion and sea inundation have been interpreted as key factors associated with allopatric speciation responsible for high levels of beta diversity across other complex islands such as New Guinea and Cuba (Heads 2001, 2002a, 2002b, 2003; Glor *et al*. 2004).

Limited evidence suggests that the Hispaniolan land mammal fauna may also have displayed distributional patterns reflecting the island's geotectonic history. Woods (1989) described a genus and species of extinct capromyid rodent, *Rhizoplagiodontia lemkei*, which was apparently restricted to the Massif de la Hotte, and Ottenwalder (2001) classified Hispaniolan solenodon (*Solenodon paradoxus*) populations from the northern and southern palaeo-islands as distinct subspecies on the basis of morphometric data. Morphological variation has also been reported in the second extant Hispaniolan land mammal, the Hispaniolan hutia (*Plagiodontia aedium*). However, the taxonomic history of the Hispaniolan hutia is confusingly complex, and several different phylogenetic and biogeographic hypotheses have been proposed to account for real or perceived patterns of morphological variation in the genus.

Originally described by Georges Cuvier in 1836 from a specimen of unknown locality within Hispaniola, *P. aedium* was then thought to be possibly extinct for most of the nineteenth and early twentieth centuries (Tate 1948), and material

recovered from prehistoric Amerindian middens was interpreted as being conspecific with *P. aedium* (Miller 1916). When living Hispaniolan hutia individuals were discovered in 1923 from Samaná Bay (Guarabo and Jovero), these proved to be smaller in size to the available zooarchaeological material, and Miller (1928) interpreted relatively subtle soft-tissue differences between Cuvier's holotype of *P. aedium* and the living Samaná population as being species-level differences, describing the latter animals as the new species *P. hylaeum*. This first phylogenetic hypothesis proposed that large-bodied (*aedium*) and small-bodied (*hylaeum*) species of *Plagiodontia* occurred sympatrically across Hispaniola, with *P. aedium* now possibly extinct.

However, a living hutia similar in size to *P. hylaeum* from southwestern Haiti (Miragoâne) was interpreted as matching Cuvier's holotype of *P. aedium*, and the large prehistoric *Plagiodontia* bones were redescribed as the extinct species *P. ipnaeum* (Johnson 1948). This second phylogenetic hypothesis proposed that morphologically similar species of hutia occurred allopatrically in different regions of Hispaniola, reflecting either the north-south palaeo-island division or Bond's Line. This hypothesis was modified by Anderson (1965), who reassessed available Hispaniolan hutia specimens and considered that relatively few characters could consistently group them into two taxa; all living Hispaniolan hutia populations were interpreted as being conspecific, but the allopatric model was explicitly maintained by recognising the Haitian subspecies *P. aedium aedium* and the Dominican Republic subspecies *P. aedium hylaeum*. Woods and Howland (1979) further claimed that no craniodental characters could consistently separate any populations of Hispaniolan hutia, therefore proposing the third phylogenetic hypothesis that all living Hispaniolan hutia are conspecific with no recognisable subspecies. However, these authors provided no published data to support this hypothesis, and Woods (1981) later suggested that different behavioural habits may be shown between geographically diverse populations of hutia across Hispaniola. This confusion over the validity of *P. hylaeum* and the identity and relationship of living hutia populations across Hispaniola has led some authors to refer to living Hispaniolan hutias simply as '*Plagiodontia* sp.' (Salazar 1977).

An extensive genetic analysis of Hispaniolan hutia populations from both Haiti and the Dominican Republic, encompassing material previously assigned to both *Plagiodontia aedium* and *P. hylaeum*, is therefore required to clarify the taxonomy, systematics and phylogenetic structuring of hutias across Hispaniola and to test the model of allopatric differentiation of the island's terrestrial biota. Identifying the presence of evolutionarily distinct mammal populations across the island is also a pressing need before targeted conservation action can be effectively implemented. However, given the difficulty of obtaining extensive modern samples of this rare and cryptic mammal, such research will be forced to utilise historical samples of varying quality in tandem with more recent material.

4.4 Materials and methods

4.4.1 Samples

A total of 41 Hispaniolan hutia samples were obtained from fieldwork in the Massif de la Hotte (Haiti) and the Sierra de Baoruco (Dominican Republic) between 2005 and 2007, and from historical museum collections (Florida Museum of Natural History; Museum of Comparative Zoology, University of Harvard; United States National Museum) (*Table 4.1*). The collection locations of these samples are distributed across both the northern and southern palaeo-islands and both east and west of Bond's Line (*Figure 4.2*). Wider geographic coverage would be desirable, but due to the restricted availability of samples and the highly threatened status of the species the samples in this study represent the maximum coverage currently available.

Figure 4.2 *Map of Hispaniola sample sites*
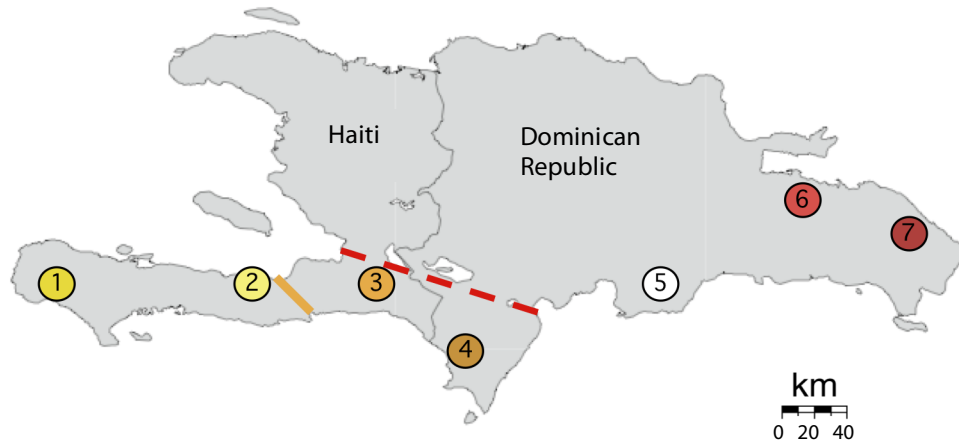
Source localities of Plagiodontia specimens are as follows: (1) Massif de la Hotte, (2) Miragôane, (3) Massif de la Selle, (4) Sierra de Baoruco, (5) San Cristobal, (6)El Jovero, (7) Guarabo. Bond's Line is shown in yellow, and the north-south palaeo-island divide is shown as a broken red line.

Table 4.1 *Island locations and sample details*

Source	Species	Country	Locality	SN	Palaeo-island	Bond's Line	N
FLMNH	P. aedium	Dominican Republic	San Cristobal	5	north	-	1
FLMNH	P. aedium	Dominican Republic	Guarbo	7	north	-	2
MCZ	P. hylaeum	Dominican Republic	Guarbo	7	north	-	5
USNM	P. aedium	Dominican Republic	El Jovero	6	north	-	10
USNM	P. aedium	Haiti	Miragôane	2	north	west	1
FC	P. aedium	Haiti	Massif de la Hotte	1	south	west	17
FC	P. aedium	Dominican Republic	Sierra de Baoruco	4	south	east	3
FLMNH	P. aedium	Haiti	Massif de la Selle	3	south	east	2

Legend: FLMNH = Florida Museum of Natural History; MCZ = Museum of Comparative Zoology, University of Harvard; USNM = United States National Museum; FC = recent field collection; SN = site number (see Figure 4.2); N = number of samples.

4.4.2 DNA extraction

Due to variation in preservation and quality of the samples, all DNA extractions were conducted in a dedicated ancient DNA laboratory, physically separated from the post- PCR laboratory. Bone samples were ground using a freezer mill (6750 SPEX CertiPrep) and liquid nitrogen and extracted using silica spin columns based on Yang *et al.* (1998) with the inclusion of 1M urea in the extraction buffer. Tissue and other non-bone samples were extracted using QIAamp DNA Micro kit and protocols. Mitochondrial DNA (mtDNA) was amplified using overlapping fragments spanning the entire cytochrome b region (1140 bp). The number of overlapping fragments used ranged from four to six, dependent on the quality of the DNA. Primer pairs were designed specifically for this study, each pair amplifying between 200 and 870 base pairs (*Appendix J)*. PCR reactions were performed using a final concentration of 1 x PCR buffer, 0.2μM of each primer, 250μM dNTPs, 2mM $MgSO_4$, 1mg/ml BSA, 1 Unit Platinum Taq DNA polymerase high fidelity, purified water, and 2μl of DNA extract in a 25μl mix. PCR conditions were 5 min at 95°C, followed by 40 cycles (increased to 55 cycles with low quality samples) of 1 min at 92°C, 1 min at 48–54°C (dependent on primer pair specifications), 1 min at 68°C, and with a final extension of 5 min at 68°C.

Amplicons were purified using Exonuclease I and Shrimp Alkaline Phosphatase. Sequencing reactions were performed by Macrogen (Korea) using a high throughput genetic analysis sequencer, ABI3730XL. Sequencing chromatograms were assembled and analysed using Sequencher 4.0 analysis software (Gene Codes Corporation). Standard ancient DNA protocols (Gilbert *et al.* 2005) were followed throughout these extraction procedures to prevent contamination, with repeated PCR amplification and sequencing of fragments to ensure DNA authenticity and the absence of miscoding lesions.

4.4.3 DNA analyses

Hispaniolan hutia sequences were manually aligned in Se-AL 2.0 with a Cuban hutia (*Capromys pilorides*) sequence from GenBank (Accession Number AF422195). Sequence diversity summary statistics were generated with samples

allocated to groups based on their geographical location. Two sets of allocations were investigated. The first approach divided samples between northern and southern palaeo-islands. The second approach further subdivided the southern population east and west of Bond's Line. Summary statistics calculated included number of haplotypes, transitions and transversions, and nucleotide and haplotype diversity. Pairwise estimates of average population sequence divergence under a Kimura-2 Parameter (K2P) model (Kimura, 1980) were also calculated in Arlequin Ver. 3.11 (Excoffier *et al.* 2005).

4.4.4 Phylogenetic analyses

Phylogenetic relationships were estimated using Maximum Likelihood (ML) and Bayesian methods. The DNA substitution model selected with ModelTest3.7 (Posada, Crandall, 1998) under Hierarchical Likelihood Ratio Tests (hLRTs) was Tamura-Nei (TrN) with gamma distribution (shape parameter 0.0078). Maximum Likelihood trees were generated in PAUP* (Swofford, 2000) using a full heuristic search, based on branch swapping with tree-bisection-reconnection (TBR). Bayesian trees were constructed using MrBayes 3.1 (Ronquist, Huelsenbeck, 2003) implementing nucleotide substitution model General Time Reversible with gamma distribution (GTR+G), using four chains (three heated one cold) were run for one million generations with a burn-in period of 1000 trees. Nodal support was determined using ML bootstrap analysis with 1000 replicates in PAUP* and by approximate posterior probabilities performed in MrBayes. A Cuban hutia was utilized as the outgroup for both sets of analyses. Connection lengths and alternative links for a minimum-spanning network of haplotypes were generated in Arlequin to visualize sequence diversity. Divergence estimates were calculated in BEAST (Drummond, Rambaut, 2007) using a GTR substitution model, a strict molecular clock and a mutation rate of 4% per million years, a moderate rodent mutation rate for cytochrome *b* (Hadly *et al.*, 2004; Irwin *et al.*, 1991). A further analysis assessed whether divergence of the north and south lineages occurred simultaneously with the unification of the north and south palaeo-islands, utilising a fixed divergence date of 10 Myr to estimate mutation rate. In all analyses chain length was set to 10, 000 000, data collected every 1000 with a burn-in of 10,000.

4.4.5 Coalescent modelling

To test the robustness of the phylogeographic inferences two plausible demographic models of Hispaniolan hutia demographic history were explored. Datasets for each model were simulated in Serial SimCoal (SSC; Anderson *et al.* 2005) and parameters were estimated by approximate Bayesian computation (ABC) (Beaumont *et al.* 2002). These models were then compared using approximate Bayes factors (see Ray *et al.* 2010) and summary statistics of the observed data were compared to those of the model datasets (with their associated parameter value estimates), using method proposed by Voight *et al.* (2005).

The first model (M1) assumed two populations (representing the north and south palaeo-islands) that diverged from a common ancestral population, of size equal to that of the north palaeo-island, 1666667 generations ago, equivalent to 10 Myr ago (reflecting the approximate timing of docking of the northern and southern palaeo-islands), and assuming a generation time for the hutia of six years (Nowak 1999; Kleinman *et al.* 1979). The two populations were assumed to have constant but unknown female effective sizes (Nef; values between 1 and 500,000 were explored independently for each). In addition bidirectional background migration was assumed since the two populations split from a common ancestral population (values of 0 to 0.00005 per generation were explored).

The second model (M2) was the same as the first except that three populations were assumed (representing the north palaeo-island and the two parts of the south palaeo-island; east and west of the Bond's line). The 'west of Bond's line' population was assumed to have branched off from the 'east of Bond's line' population 1666661 generations ago, which in turn was assumed to have branched off from the 'north palaeo-island' population 1666667 generations ago. All three populations were assumed to be of constant but unknown female effective sizes (Nef; values between 1 and 500,000 were explored independently for each). Bidirectional background migration was assumed between the 'west of Bond's line' and the 'east of Bond's line' populations (values between 0 and 0.0005 per generation were explored), and between the 'east of Bond's line' and the 'north palaeo-island' populations (values between 0 and 0.00005 per generation were explored). For both models a mutation rate of 4% per million years (Irwin *et al.*

1991; Hadly *et al.* 2004), a transition: transversion rate of 17.1:1, and rates to be gamma distributed with parameter 0.094 and 4 rate classes were assumed. For both models 4.5 million coalescent simulations were performed.

Sequences were sampled from the coalescent simulations according to the number of sequences generated in this study. In the case of model 1, two samples were taken from the 'south palaeo-island' simulated population (n = 5 and 11) and one from the 'north palaeo-island' simulated population (n = 11). Within sample (number of haplotypes, number of segregating sites, average pairwise difference, haplotype diversity (biased by n-1/n) and Tajima's D) and between sample (number of private haplotypes to each sample, average pairwise difference, F_{ST}) summary statistics were calculated on the observed data using Arlequin software. Model parameters were estimated by approximate Bayesian computation (ABC) (Beaumont *et al.* 2002), conditioning on the same summary statistics as listed above.

Marginal posterior distributions were then estimated for the parameters of each model. The approximate joint posterior distribution was first obtained by performing a local-linear multivariate weighted regression adjustment (see Beaumont *et al.* 2002), from which the marginal posterior distributions were derived for each parameter.

In brief (see Beaumont *et al.* 2002 for details), comparisons between the two models where made by calculating the Euclidean distance between the observed and simulated summary statistics for each of the 9 million simulated datasets. With tolerance proportion $F_{\delta} = 0.001$ the 4,500 best-fitting parameter sets from each of the two models were retained, and placed into a common pool. These 9,000 simulations were then ordered by increasing Euclidean distance, which was recalculated after normalization of the summary statistics using means and standard deviations computed on the pool. The relative proportion of simulations from each model in the *n*-smallest Euclidean distances gave an estimate of the posterior probability of each model (Estoup *et al.* 2004, Ray *et al.* 2010).

In the above analysis a two, and a three-population model were assumed to be the simplest models that reflect the biogeographical structuring of Hispaniolan hutia populations over the last 10 million years. Nonetheless, it is possible that misspecified models have been used – which would lead to misleading parameter estimates. To examine this possibility Fisher's method was applied to combine 2-tailed probabilities of the observed conditioning statistics – obtained by comparison to simulation using the modes of the parameter estimates obtained by ABC for the best model. The resultant chi^2 values were compared to those obtained by comparing each simulation against the set of all other simulations for each model (Voight *et al.* 2005).

4.5 Results

4.5.1 DNA sequence data

From the 41 Hispaniolan hutia individuals sampled, 27 successfully yielded mitochondrial DNA for the entire cytochrome *b* region. From those 27 sequences a total of 18 haplotypes were identified encompassing six out of the seven sampling localities under investigation; the sample from San Cristobal (location 5) did not yield viable mtDNA. Haplotype diversity indices were high (0.70–0.95) for each of the regions analysed. However, nucleotide diversity was markedly lower in the northern population (0.001) compared to the southern populations (south eastern 0.016, south western 0.013, *Table 4.2*).

Table 4.2 *Molecular diversity*

Region	N	H	ti	tv	h	π
north palaeo-island	11	7	6	0	0.8182 (0.1191)	0.001085 (0.000837)
south palaeo-island (east of 'Bond's Line')	5	3	40	4	0.7000 (0.2184)	0.016491 (0.010342)
south palaeo-island (west of 'Bond's Line')	11	8	42	2	0.9455 (0.0535)	0.012855 (0.007041)
south palaeo-island (entire)	16	11	53	4	0.9500 (0.0364)	0.018604 (0.009710)

Molecular diversity indices (N = individuals, H = haplotypes, ti = transitions tv = transversions, h = haplotype diversity, π = nucleotide diversity, parentheses = standard error)

The genetic differences between these regionally differentiated populations calculated using corrected K2P parameters and pairwise differences indicate low level divergence between populations grouped east and west of Bond's Line, with increased divergence percentages when the northern palaeo-island population is compared to the southern populations (*Table 4.3*).

Table 4.3 *Population average pairwise estimates of sequence divergence with Kimura-2 Parameter*

	north palaeo-island	south palaeo-island (east of 'Bond's Line')	south palaeo-island (west of 'Bond's Line')	south palaeo-island (entire)
north palaeo-island		2.87%	3.20%	2.86%
south palaeo-island (east of 'Bond's Line')			1.03%	

4.5.2 Phylogenetic analyses

Maximum Likelihood and Bayesian analyses generated highly congruent phylogenetic trees, thus only the Bayesian tree is shown, with both the Bayesian approximated posterior probabilities and ML bootstrap values indicated (*Figure 4. 3*). The inclusion of a minimum spanning network (*Figure 4.4*) provides a further comparative representation between geographically distinct Hispaniolan hutia populations, with the southern population exhibiting a far greater diversity among haplotypes. All phylogenetic analyses recovered the same two monophyletic clades with strong statistical support values. The northern clade contains seven haplotypes (11 samples) from two localities, and the southern clade is represented by 11 haplotypes (16 samples) from four localities (two localities on either side of Bond's Line). The southern clade exhibits a further divisional trend with the preponderance of samples separating into subclades according to location, east or west of 'Bonds line'. Interestingly, the two samples that did not conform to this trend (South West 2 and South East 3) were collected from sites proximate to 'Bond's Line', site three to the east and site two to the west.

Figure 4.3 *Phylogeny for the Hispaniolan hutia*

Bayesian tree constructed in MrBayes with GTR+G for 1000,000 generations, using hutia haplotypes. Nodal support is shown through approximate Bayesian probabilities above the line and bootstrap values (PAUP*) in parentheses (only values above 50% shown).Cuban hutia used as an outgroup (not shown). Colours and numbers indicate the sample locations from Figure 4.2, each colour block is a sample, haplotypes that represent multiple samples are indicated by multiple blocks

Figure 4.4 *Minimum spanning network for the Hispaniolan hutia*
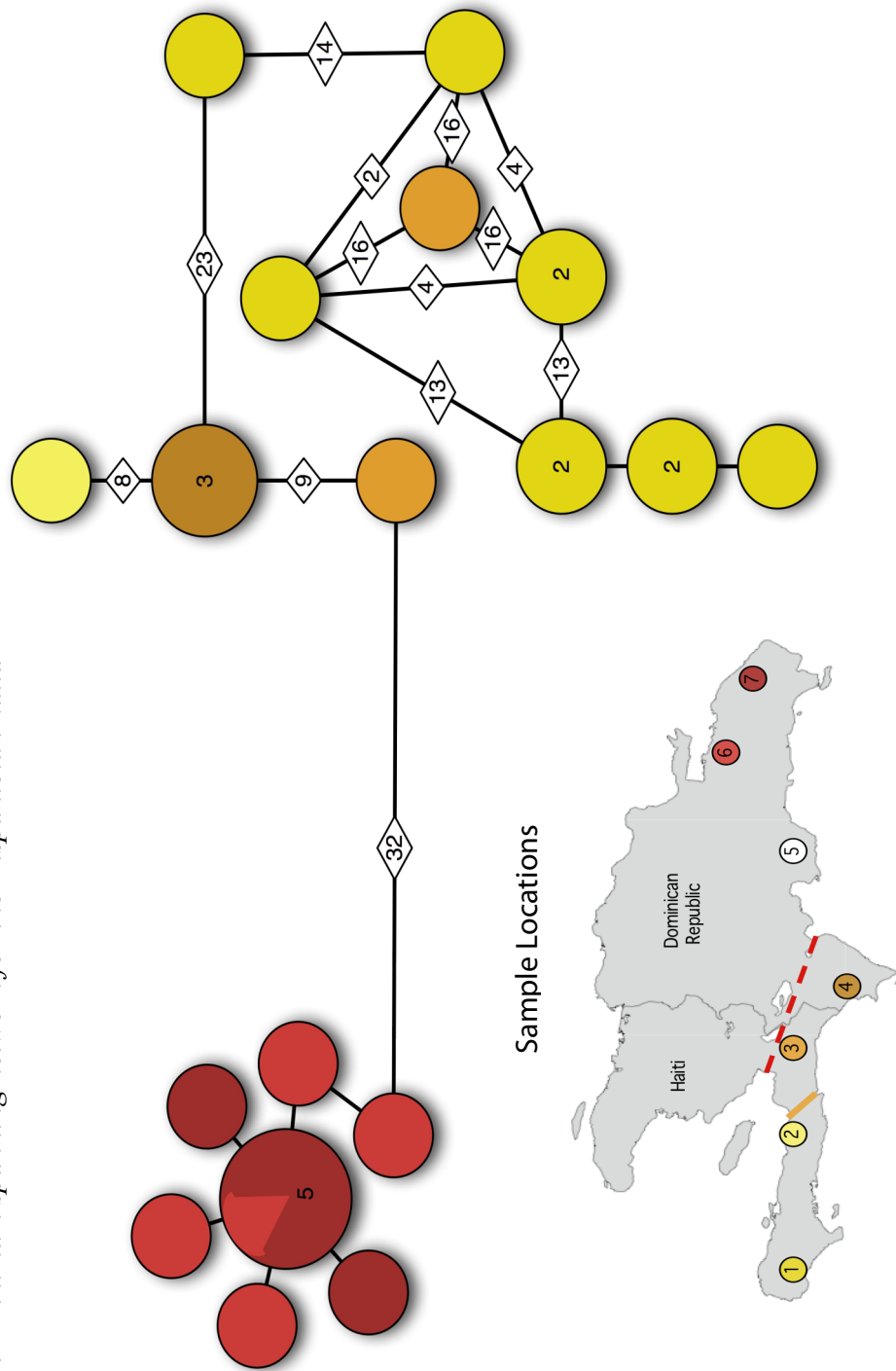
Sample Locations

Calculated in Arlequin. The numbers inside circles are the total number of samples represented by the haplotype. The numbers inside diamonds are the number of connection lengths. Colours indicate sample collection locations from Figure 4.2

The results from the divergence date estimates indicate lineage separation of north and south palaeo-islands occurred during the Pleistocene approximately 0.5 Myr ago with the east west division in the southern palaeo-island occurring approximately 0.4 Myr ago (*Table 4.4*).

When the divergence date for the north and south hutia populations was fixed to 10 Myr ago, the mean mutation rate required was estimated to 0.21 % per million years, (95% HPD 0.156 – 0.260).

Table 4.4 *Population divergence dates*

	Divergence Dates (Myr)		
	95% HPD lower	95% HPD upper	Mean
north and south palaeo-islands	0.408	0.659	0.534
east and west of Bond's Line	0.277	0.466	0.365

4.5.3 Coalescent modelling

Prior values of Nef and migration rates for those simulations that best fit the actual data were obtained using ABC and posterior probability distributions for those are shown in *Figures 4.5* and *4.6*. Model one (northern and southern palaeo-island model; *Figure 4.5*) and model two (northern, south eastern and south western palaeo-island model; *Figure 4.6*) with the mode and quantiles for each (2.5% and 97.5%) shown in *Table 4.5*. The south western population has the highest estimated population size with a modal number of 16139 females with 97.5% confidence intervals of 5091 to 91085. The estimated migration rates from both models and across both boundaries were low, the highest migration rate was from the south eastern population to the south western population, with 97.5% confidence intervals of 0.0000111 to 0.0002778 modal rate estimated at 0.0000457.

Figure 4.5 *Posterior probability distributions of Nef and migration rate for model 1 (two populations)*
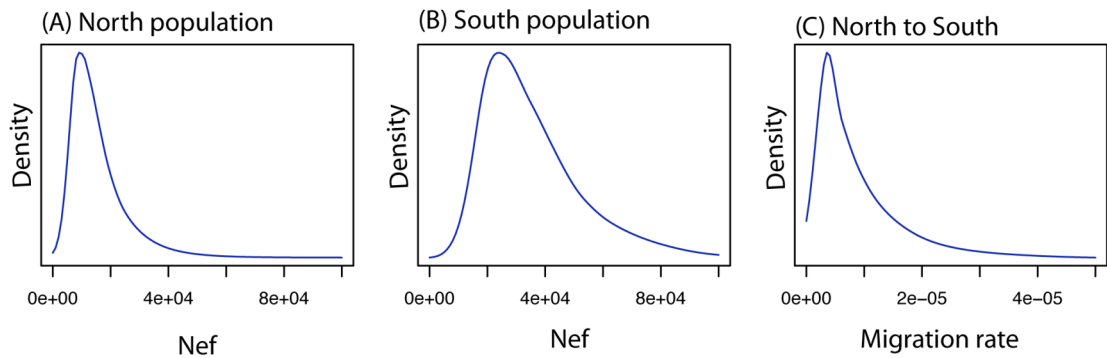
Posterior probability density curves: Female effective population size in (A) the northern population and (B) the southern population and (C) the migration rate from the northern to the southern populations

Figure 4.6 *Posterior probability distributions of Nef and migration rate for model 2 (three populations)*
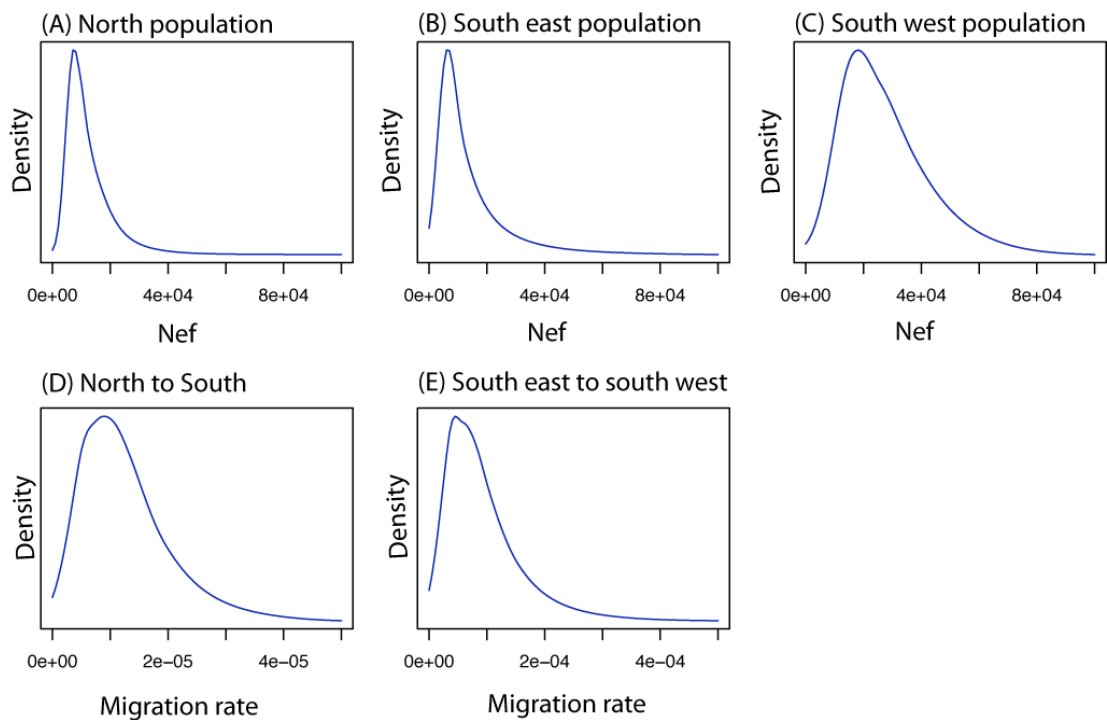
Posterior probability density curves: Female effective population size in (A) the northern population (B) the south eastern population (C) the south western population. Migration rates from (D) the northern to the south eastern populations and (E) the south eastern to the south western populations

Table 4.5 *Parameter quantiles (2.5%, 97.5%) and modal values*

	Population	Parameter	Quantile		Mode
			2.50%	**97.50%**	
Model 1	North	Nef	3888	40848	9192
	South	Nef	11957	83438	23909
	North to south	Migration rate (per generation)	0.0000008	0.0000336	0.0000037
Model 2	North	Nef	3112	35393	7477
	South east	Nef	1650	56207	6509
	South west	Nef	5849	66407	18086
	North to south east	Migration rate	0.0000017	0.0000337	0.0000090
	South east to south west	Migration rate	0.0000111	0.0002778	0.0000457

Quantile values and mode of the posterior probability distribution for Nef and migration rate

Figure 4.7 *The approximated posterior probability for models 1 and 2*
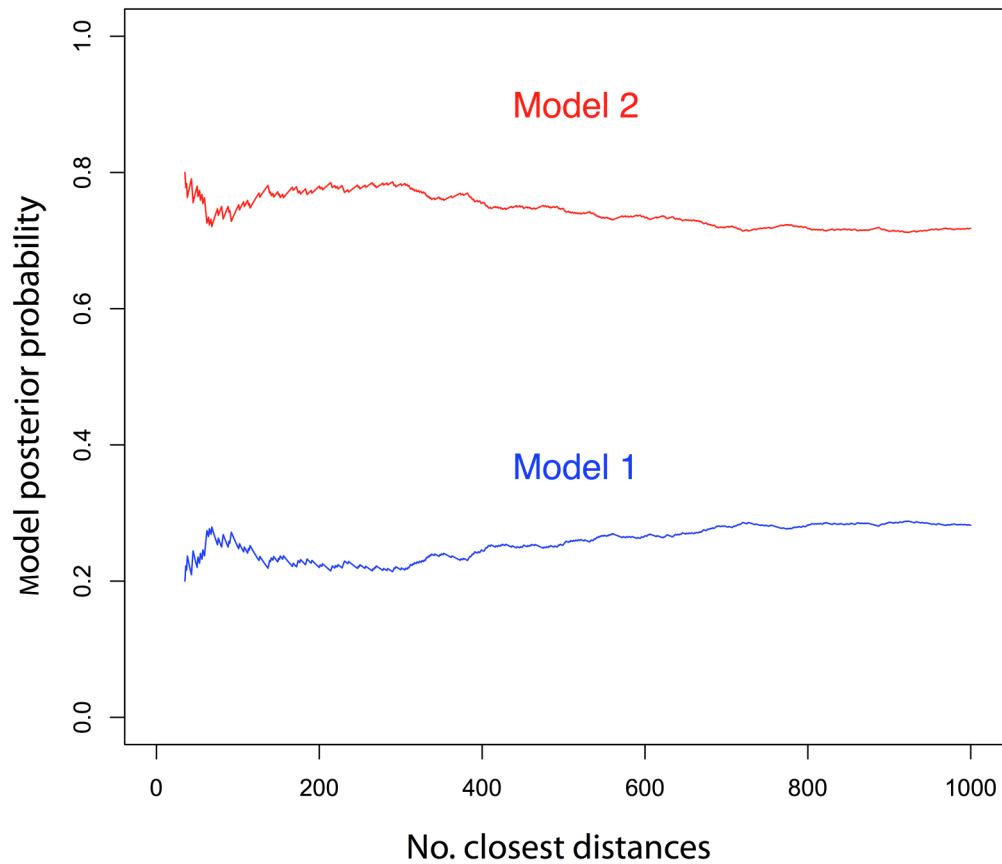
The blue line indicates posterior probability values for the two population model (model 1). The red line indicates posterior probability values for the three population model (model 2). Analysed using Bayes factor.

The results from the Bayes factor analysis show greater support, through higher posterior probability values, for the three-population model (model 2), when compared to the two-population model (model 1; *Figure 4.7*). The three-population model (model 2) was further supported by the Voight method, returning a two-tailed p-value of 0.9173. This indicates that the observed data is typical for data generated by the model and, as such, the model is an appropriate model to use.

4.6 Discussion

The results demonstrate a high level of phylogenetic structure within the Hispaniolan hutia, which is biogeographically consistent with the geotectonic history of the island. The major phylogenetic division within the genus is between individuals from the northern and southern palaeo-islands, represented by two distinct monophyletic clades (*Figures 4.3 & 4.4*). A further, non-absolute subdivision of the southern population across the Jacmel-Fauché depression into eastern and western lineages is also apparent.

SSC analyses simulated a range of migration values between hutia populations, but appropriate model results (those close to the observed phylogenetic data) were only achieved when extremely low migration rates were parameterised (*Figures 4.5 & 4.6*). The ecology of Hispaniolan hutia is poorly understood, but it is thought to exist in family-based social groups (Radden 1967; Woods 1981) similar to other hutia species (Anderson *et al.*, 1983; Clough, 1972), suggesting that it is not a solely male-dispersal strategist. Thus, while our analyses are based on mtDNA, and therefore represent female history exclusively, our analyses should be valid for the species as a whole. Using migration as a proxy for isolation, hutia populations from the three biogeographic regions of Hispaniola are almost completely genetically isolated from each another. In the three population model estimated migration rates between the north and south eastern populations were negligible, with a 97.5% confidence intervals of 0.0000017 to 0.0000337 and a modal rate estimated at 0.0000090. Migration across the Jacmel-Fauché depression was estimated at a higher rate, with a 97.5% confidence interval of 0.0000111 to 0.0002778 and a modal estimated rate of 0.0000457, this still constitutes a minimal level of migration between populations. Population division across Bond's Line is further supported by comparative Bayes factor analyses (*Figure 4.7*), where the more progressive model incorporating three distinct lineages with two migration matrices had greater posterior probability support than the simpler two-lineage model.

The pattern of historical allopatric lineage divergence observed in this study across the northern and southern palaeo-islands of Hispaniola is spatially

congruent with that shown from other terrestrial vertebrates (Gifford, Larson, 2008), and further illustrates the importance of *in situ* vicariant processes in driving differentiation and lineage accumulation on large islands. However, this spatial congruence is not matched by temporal congruence in lineage divergence in the hutia or other Hispaniolan taxa for which phylogeographic studies have been conducted. Divergences between northern and southern populations of other Hispaniolan terrestrial vertebrates have been dated to the Miocene (chat-tanagers: 9.7 Myr) or Pliocene (teiid lizard: 4.3 Myr). The chat-tanager (Townsend *et al.* 2007) and the teiid lizard (Gifford, Larson 2008) studies employ a multilocus coalescent-based approach to estimate timing of the divergence between northern and southern populations. Townsend *et al.* (2007) utilise a range of estimated priors for mutation rate, while Gifford and Larson (2008) calibrate the mutation rate based on a previous divergence estimate, calibrated through immunological distance analysis (Hower, Hedges 2003). Accurate calibration is a key component when estimating divergence dates and different methods can produce varying results. However, the estimated divergence dates for the chat-tanager and teiid lizard would require significant errors in mutation rate estimates for the reported dates to be temporally aligned.

Results from this study indicate that divergence between northern and southern hutia populations occurred appreciatively more recently, between 0.408 and 0.659 Myr (95% HPD) with a mean estimated value of 0.534 Myr (*Table 4.4*). While the mutation rates utilised in this study and in previous analyses (Townsend *et al.* 2007; Gifford, Larson 2008) are considered plausible for the species and gene under investigation, an erroneous mutation rate can result in an erroneous estimation for the date of divergence. However, for the divergence of the northern and southern hutia populations to be temporally congruent with the unification of the north and south palaeo-islands, ca. 10 Myr, this would necessitate a mutation rate of 0.21 % per million years. This is an order of magnitude slower than a standard mutation rate for the cytochrome *b* region, thus a divergence that is temporally congruent with the ancient docking of the palaeo-islands is rejected as implausible.

The variation in phylogeographic divergence dates across the main geotectonic division of Hispaniola indicates that the marine channel between the northern and southern palaeo-islands, and subsequent inundation of the Neiba Valley throughout the Neogene and Quaternary, acted as a semi-permeable barrier to gene flow that permitted periodic colonisation events to occur throughout its geological history either via overwater dispersal or periodic regional marine regression. Similar patterns of asynchronous, progressive colonisation across marine barriers have also been demonstrated for the faunal history of other island systems such as Madagascar (Poux *et al*. 2005; Ali, Huber 2010).

The more recent divergence by Hispaniolan hutia across the north-south palaeo-island boundary may also reflect ecological differences between this mammal and the other Hispaniolan taxa for which phylogeographic data are available, both of which have restricted elevational distributions. Chat-tanagers are high-elevation dense forest specialist with a disjunct modern-day distribution across Hispaniolan mountain ranges (Latta *et al*. 2006; Townsend *et al*. 2007), and the teiid lizard occurs only in the xeric lowlands of Hispaniola (Gifford, Larson 2008). In contrast, the Hispaniolan hutia is apparently an ecological generalist; although its current distribution and habitat preferences remain poorly understood, it has been recorded across a range of habitat types and elevations from mangrove swamp to montane forest during the past century (Miller 1928; Woods 1981; Sullivan 1983; Woods *et al*. 1985; Turvey *et al*. 2008). This wide habitat tolerance may have provided the hutia with a greater ecological flexibility for occupying and colonising habitats on either side of the Neiba Valley throughout periods of environmental change across the Neogene and Quaternary, making Hispaniola's geotectonic and biogeographic boundaries less likely to act as significant barriers to gene flow over geological time. However, this hypothesis may be challenged by the different patterns of phylogenetic distinctiveness shown by southern palaeo-island populations of the Hispaniolan hutia and chat-tanagers; whereas the Hispaniolan hutia contains distinct eastern and western clades, chat-tanager populations exhibit no genetic distinction across Bond's Line (Townsend *et al*. 2007).

Following the confused taxonomic history of the Hispaniolan hutia, the identification of three phylogenetically distinct allopatric hutia lineages provides an important opportunity to clarify the taxonomy of the genus. The analysis of cytochrome b sequence divergence within the Capromyidae by Woods *et al.* (2001) found intraspecific levels of sequence divergence using pairwise estimates with Kimura-2 parameter between different subspecies of the Cuban hutia ranged from 0.4 – 1.8%, and that interspecific levels of sequence divergence across the family ranged from 3.0 – 20.4%. Results from this study (*Table 4.3*) estimate sequence divergence between northern and southern hutia populations at 2.86%, and between southern populations either side of Bond's Line at 1.03%. The strict monophyly of the northern and southern populations provides some support for species-level distinction; however, the level of sequence divergence between these lineages would constitute the lowest interspecific divergence value recorded for this family (Woods *et al.* 2001).

In the absence of supporting data on morphometric variation between these populations, I therefore conservatively recognise a single living species of hutia (*Plagiodontia*) on Hispaniola, with maintenance of two phylogenetically distinct subspecies, *P. aedium aedium* Cuvier, 1836 (southern lineage) and *P. aedium hylaeum* Miller, 1928 (northern lineage) are maintained. The estimated divergence between the two southern populations falls within the range of intraspecific divergence estimates for the Cuban hutia, and in the absence of supporting data on morphometric variation between these populations, a single taxon is recognised for the entire southern palaeo-island.

The recognition of distinct Hispaniolan hutia lineages is an important process not only for taxonomic clarification but also for conservation prioritization. Hutia represent one of the last two surviving endemic land mammals on Hispaniola and is now classified as Endangered by IUCN (2008), so warrants significant conservation concern. Hispaniola's different biogeographic regions are currently experiencing differing levels of anthropogenic habitat loss, which reaches levels of >95% across much of Haiti (Sergile, Woods 2001; Latta 2005). Despite the conservative taxonomic approach taken in this study it is important to stress that

the three hutia populations identified should be treated as distinct evolutionary units.

Genetic analyses provide further important implications for conservation by highlighting striking disparities both in within-clade phylogenetic structure and in estimated effective population size between different Hispaniolan hutia subspecies and populations. Whereas the southern population exhibits high levels of nucleotide diversity, with up to 23 mutational steps between neighbouring haplotypes, the northern population exhibit very low nucleotide diversity with a maximum of three mutational steps between all haplotypes (*Figure 4.4*). Although all northern samples - as a result of sampling constraints - are from a relatively restricted part of the north eastern Dominican Republic, they still represent two sampling localities situated approximately 20 km apart (Miller 1928). Similarly, the posterior probability distribution of Nef in the northern population are low, estimates using the better supported three population model (model 2) with a 97.5% confidence interval of 3112 to 35393 predict a modal value of 7447 females (*Table 4.5*).

Low levels of nucleotide diversity can indicate either a past population bottleneck or an ongoing population decline, it is therefore concerning that the northern palaeo-island samples which exhibit such reduced genetic diversity are all old historical samples collected in the 1920s. The lowlands of the eastern Dominican Republic were more densely settled by Amerindians than other parts of Hispaniola before European contact (Wilson 2007), and extensive Amerindian exploitation of Hispaniolan hutia and other now-extinct hutia is evidenced by early historical records and zooarchaeological analysis of kitchen middens (Miller 1929; Johnson 1948). The observed reduction in genetic diversity in the northern hutia subspecies may therefore represent a pre-European population bottleneck or decline. There is little information available on the current status of hutia across much of the northern palaeo-island, and so further survey work to establish the distribution and abundance of the northern subspecies should be a conservation priority.

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In contrast, although almost all of the samples from the south western population are from a similarly restricted geographical area (the Duchity region of the Massif de la Hotte; Turvey *et al*. 2008), neighbouring haplotypes from individuals in this region vary by as much as 14 mutational steps (*Figure 4.4*). This population has the highest estimated population size with a modal number of 16139 females with 97.5% confidence intervals of 5091 to 91085 (*Table 4.5*). The high level of haplotype diversity shown by hutia from the Massif de la Hotte is reflected across wider levels of biodiversity, as this region is a globally important biodiversity hotspot containing extremely high levels of range-restricted endemic species (Ricketts *et al.*, 2005). Historically the region may have been more inaccessible to human encroachment than lowland parts of Hispaniola, and it probably represented a refugium for mesic forest-dwelling species during Quaternary environmental fluctuations (MacPhee *et al.*, 2000), as well as providing a wider range of local environments that could have driven *in situ* diversification. Efforts to maintain this healthy hutia population and the wider ecosystems of the Massif de la Hotte should therefore also represent an urgent conservation priority.

4.7 References

Ali JR, Huber M (2010) Mammalian biodiversity on Madagascar controlled by ocean currents. *Nature*, **463**, 653-656.

Anderson CNK, Ramakrishnan U, Chan YL, Hadly EA (2005) Serial SimCoal: a population genetics model for data from multiple populations and points in time. *Bioinformatics*, **21**, 1733-1734.

Anderson S (1965) Conspecificity of *Plagiodontia aedium* and *P. hylaeum* (Rodentia). *Proceedings of the Biological Society of Washington*, **78**, 95-98.

Anderson S, Woods CA, Morgan GS, Oliver WLR (1983) *Geocapromys brownii*. *Mammalian Species*, **201**, 1-5.

Beaumont MA, Zhang WY, Balding DJ (2002) Approximate Bayesain computation in population genetics. Genetics **162**, (4), 2025-2035

Clough GC (1972) Biology of the Bahaman hutia, *Geocapromys ingrahami*. *Journal of Mammalogy*, **53**, 807-823.

Condis Fernández MM, Jiménez Vásquez O, Arredondo C (2005) Revisión taxonómica del género *Nesophontes* (Insectivora: Nesophontidae) en Cuba: análisis de los caracteres diagnóstico. *Monografies de la Societat d'Història Natural de les Balears*, **12**, 95-100.

Díaz-Franco S (2001) Situación taxonómica de *Geocapromys megas* (Rodentia: Capromyidae). *Caribbean Journal of Science*, **37**, 72-80.

Drummond AJ, Rambaut (2007) A. BEAST: Baysian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7. 214-222.

Estoup A, Beaumont M, Sennedot F, Moritz C, Cornuet J-M (2004) Genetic analysis of complex demographic scenarios: Spatially expanding populations of the cane toad, Bufo marinus. *Evolution* **58**, 2021-2036

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47–50.

Gifford ME, Powell R, Larson A, Gutberlet RL Jr (2004) Population structure and history of a phenotypically variable teiid lizard (*Ameiva chrysolaema*) from Hispaniola: the influence of a geologically complex island. *Molecular Phylogenetics and Evolution*, **32**, 735-748.

Gifford ME, Larson A (2008) *In situ* genetic differentiation in a Hispaniolan lizard (*Ameiva chrysolaema*): a multilocus perspective. *Molecular Phylogenetics and Evolution*, **49**, 277-291.

Gilbert MTP, Bandelt H-Jr, Hofreiter M, Barnes I (2005) Assessing ancient DNA studies. *Trends in Ecology and Evolution*, **20**, 541-544.

Glor RE, Gifford ME, Larson A, Losos JB, Rodriguez Schettino L, Chamizo Lara AR, Jackman TR (2004) Partial island submergence and speciation in an adaptive radiation: a multilocus analysis of the Cuban green anoles. *Proceedings of the Royal Society B*, **271**, 2257-2265.

Glor RE, Kolbe JJ, Powell R, Larson A, Losos JB (2003) Phylogenetic analysis of ecological and morphological diversification in Hispaniolan trunk-ground anoles (*Anolis cybotes* group). *Evolution*, **57**, 2383-2397.

Graham A (2003) Geohistory models and Cenozoic paleoenvironments of the Caribbean region. *Systematic Botany*, **28**, 378-386.

Hadly EA, Ramakrishnan U, Chan YL*,* Van Tuinen M, O'Keefe K, Spaeth PA, Conroy CJ (2004) Genetic response to climatic change: insights from ancient DNA and phylochronology. *PLoS Biology*, **2**, e290.

Heads M (2001) Birds of paradise and bowerbirds: regional levels of biodiversity in New Guinea and correlations with terrane tectonics. *Journal of Zoology*, **255**, 331-339.

Heads M (2002a) Birds of paradise, vicariance biogeography and terrane tectonics in New Guinea. *Journal of Biogeography*, **29**, 261-284.

Heads M (2002b) Regional patterns of biodiversity in New Guinea animals. *Journal of Biogeography*, **29**, 285-294.

Heads M (2003) Ericaceae in Malesia: vicariance biogeography, terrane tectonics and ecology. *Telopea*, **10**, 311-449.

Hedges SB (1999) Distribution patterns of amphibians in the West Indies. In: *Patterns of distribution of amphibians: a global perspective* (ed. Duellman WE), pp. 211-254. Johns Hopkins University Press, Baltimore.

Hedges SB (2001) Biogeography of the West Indies: an overview. In: *Biogeography of the West Indies: patterns and perspectives* (ed. Woods CA, Sergile FE), pp. 15-33. CRC Press, Boca Raton, FL.

Hedges SB (2006) Paleogeography of the Antilles and origin of West Indian terrestrial vertebrates. *Annals of the Missouri Botanical Garden*, **93**, 231-244.

Hower LM, Hedges SB (2003) Molecular phylogeny and biogeography of West Indian teiid lizards of the genus *Ameiva*. *Caribbean Journal of Science*, **39**, 298-306.

Irwin D, Kocher T, Wilson A (1991) Evolution of the cytochrome b gene of mammals. *Journal of Molecular Evolution*, **32**, 128-144.

Iturralde-Vinent MA, MacPhee RDE (1999) Paleogeography of the Caribbean region: implications for Cenozoic biogeography. *Bulletin of the American Museum of Natural History*, **238**, 1-95.

IUCN (International Union for Conservation of Nature) (2008) 2008 IUCN Red List of threatened species. IUCN, Gland, Switzerland. Available from www.iucnredlist.org (accessed August 2009).

Johnson DH (1948) A rediscovered Haitian rodent, *Plagiodontia aedium*, with a synopsis of related species. *Proceedings of the Biological Society of Washington*, **61**, 69-76.

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, **16**, 111-120.

Kleiman D, Eisenberg J, Maliniak E (1979) Reproductive parameters and productivity of caviomorph rodents. In Eisenberg JF (ed) Vertebrate ecology in the northern Neotropics. Smithsonian Institution Press, pp 173-183.

Latta S (2005) Complementary areas for conserving avian diversity on Hispaniola. *Animal Conservation*, **8**, 69-81.

Latta S, Rimmer C, Keith A, Wiley J, Raffaele H, McFarland K, Fernandez E (2006) *Birds of the Dominican Republic and Haiti*. Christopher Helm, London.

Leonard JA (2008) Ancient DNA applications for wildlife conservation. *Molecular Ecology*, **17**, 4186-4196.

Losos JB, Schluter D (2000) Analysis of an evolutionary species-area relationship. *Nature*, **408**, 847-850.

MacPhee RDE (2009) *Insulae infortunatae*: establishing a chronology for Late Quaternary mammal extinctions in the West Indies. In: *American megafaunal extinctions at the end of the Pleistocene* (ed. Haynes G), pp. 169-193. Springer.

MacPhee RDE, Flemming C (1999) Requiem æternam: the last five hundred years of mammalian species extinctions. In: *Extinctions in near time: causes,*

contexts, and consequences (ed. MacPhee RDE), pp. 333-371. Kluwer Academic/Plenum, New York.

MacPhee RDE, White JL, Woods CA (2000) New megalonychid sloths (Phyllophaga, Xenarthra) from the Quaternary of Hispaniola. *American Museum Novitates*, **3303**, 1-32.

Mann P, Draper G, Lewis JF (1991) An overview of the geologic and tectonic development of Hispaniola. In: *Geologic and tectonic development of the North America – Caribbean plate boundary in Hispaniola* (ed. Mann P, Draper G, Lewis JF), pp. 1-51. Geological Society of America Special Publication **262**.

Maurrasse F, Pierre-Louis R, Rigaud J-G (1982) Cenozoic facies distribution in the southern peninsula of Haiti and the Barahona Peninsula, Dominican Republic, and its relations concerning tectonic evolution of the La Selle-Baoruco block. *Caribbean Geology, Collected Contributions*, **9**, 1-24.

Mertens R (1939) Herpetologische Ergebnisse einer Reise nach der Insel Hispaniola Westindien. *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft*, **449**, 1-84.

Miller GS Jr (1916) Bones of mammals from Indian sites in Cuba and Santo Domingo. *Smithsonian Miscellaneous Contributions*, **66(12)**, 1-10.

Miller GS Jr (1928) The rodents of the genus *Plagiodontia. Proceedings of the United States National Museum*, **72**, 1-8.

Miller GS Jr (1929) Mammals eaten by Indians, owls, and Spaniards in the coast region of the Dominican Republic. *Smithsonian Miscellaneous Contributions*, **82(5)**, 1-16.

Nowak R. M (1999) Walker's Mammals of the World, sixth edition. The John Hopkins University Press

Ottenwalder JA (2001) Systematics and biogeography of the West Indian genus *Solenodon*. In: *Biogeography of the West Indies: patterns and perspectives* (ed. Woods CA, Sergile FE), pp. 253-259. CRC Press, Boca Raton, FL.

Posada D, Crandall K (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics*, **14**, 817-818.

Poux C, Madsen O, Marquard E, Vieites DR, de Jong WW, Vences M (2005) Asynchronous colonisation of Madagascar by the four endemic clades of

primates, tenrecs, carnivores, and rodents as inferred from nuclear genes. *Systematic Biology*, **54**, 719-730.

Radden RD (1967) The Dominican republic hutia *Plagiodontia aedium hylaeum* in captivity. Unpublished M.Sc. thesis, University of Puget Sound.

Ray N, Wegmann D, Fagundes NJR*, et al.* (2010) A Statistical Evaluation of Models for the Initial Settlement of the American Continent Emphasizes the Importance of Gene Flow with Asia. *Molecular Biology and Evolution* **27**, 337-345.

Ricketts TH, Dinerstein E, Boucher T *et al*. (2005) Pinpointing and preventing imminent extinctions. *Proceedings of the United States National Academy of Sciences*, **51**, 18497-18501.

Roca AL, Bar-Gal GK, Eizirik E, Helgen KM, Maria R, Springer MS, O'Brien SJ, Murphy WJ (2004) Mesozoic origin for West Indian insectivores. *Nature*, **429**, 649-651.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572-1574.

Salazar L (1977) Notas generales sobre *Plagiodontia* sp. con commentarios sobre los especimenes obtenidos por el ZOODOM. *ZOODOM*, **2**, 16-23.

Schwartz A (1980) The herpetogeography of Hispaniola, West Indies. *Studies on the fauna of Curaçao and other Caribbean Islands*, **61**, 86-127.

Sergile FE, Woods CA (2001) Status of conservation in Haiti: a 10-year retrospective. In: *Biogeography of the West Indies: patterns and perspectives* (ed. Woods CA, Sergile FE), pp. 547-560. CRC Press, Boca Raton, FL.

Sullivan CP (1983) Status and distribution of *Plagiodontia aedium* in the Dominican Republic. Unpublished M.Sc. thesis, University of Florida.

Swofford DL (2000) Phylogenetic analysis using parsimony (* and other methods),. Sinaur Associates, Sunderland, Massachusetts.

Tate GHH (1948) Notes on the Hispaniolan hutia, *Plagiodontia*, and extinct related genera. *Journal of Mammalogy*, **29**, 176-178.

Townsend AK, Rimmer CC, Latta SC, Lovette IJ (2007) Ancient differentiation in the single-island avian radiation of endemic Hispaniolan chat-tangers (Aves: *Calyptophilus*). *Molecular Ecology*, **16**, 3634-3642.

Turvey ST (2009) *Holocene extinctions*. Oxford University Press, Oxford.

Turvey ST, Meredith HMR, Scofield RP (2008) Continued survival of Hispaniolan solenodon *Solenodon paradoxus* in Haiti. *Oryx*, **42**, 611-614.

Voight BF (2005) Interrogating multiple aspects of variation in a full resequencing data set to infer human population size changes. *Proceedings of the National Academy of Sciences* **102**, 18508-18513

Williams EE (1961) Notes on Hispaniolan herpetology. 3. The evolution and relationships of the *Anolis semilineatus* group. *Museum of Comparative Zoology, Breviora*, **136**, 1-8.

Wilson SM (2007) *The archaeology of the Caribbean*. Cambridge University Press, Cambridge.

Woods CA (1981) Last endemic mammals in Hispaniola. *Oryx*, **16**, 146-152.

Woods CA (1989) A new capromyid rodent from Haiti: the origin, evolution, and extinction of West Indian rodents, and their bearing on the origin of New World hystricognaths. *Natural History Museum of Los Angeles County, Science Series*, **33**, 59-90.

Woods CA, Borroto R, Kilpatrick CW (2001) Insular patterns and radiations of West Indian rodents. In: *Biogeography of the West Indies: patterns and perspectives* (ed. Woods CA, Sergile FE), pp. 335-353. CRC Press, Boca Raton, FL.

Woods CA, Howland EB (1979) Adaptive radiation of capromyid rodents: anatomy of the masticatory apparatus. *Journal of Mammalogy*, **60**, 95-116.

Woods CA, Ottenwalder JA (1992) *The natural history of southern Haiti*. Florida Museum of Natural History, Gainesville, FL.

Woods CA, Ottenwalder JA, Oliver WLR (1985) Lost mammals of the Greater Antilles: the summarised findings of a ten weeks field survey in the Dominican Republic, Haiti and Puerto Rico. *Dodo*, **22**, 23-42.

Yang DY, Eng B, Waye JS, Dudar JC, Saunders SR (1998) Technical note: improved DNA extraction from ancient bones using silica-based spin columns. *American Journal of Physical Anthropology*, **105**, 539–543.

Chapter 5: Rice rat colonisation in the Lesser Antilles

5.1 Abstract

Islands provide the ideal locale to study biological evolution and adaptive radiation. One extensive, yet relatively understudied island chain is the Lesser Antilles, part of a volcanic arc that forms the West Indies. This research focuses on a key component of the region's fauna, the Antillean rice rats (Muroidea: Sigmodontinae: Oryzomyini), which became completely extinct within the recent historical period. Rice rat material has been recovered in late Holocene zooarchaeological and palaeontological contexts from most of the Lesser Antillean islands, but almost nothing remains known about this extensive evolutionary radiation. I obtained rice rat material from a total of eight Lesser Antilleans islands; samples from St. Kitts, Nevis, St. Eustatius, Martinique, St. Lucia and Guadeloupe successfully yielding rice rat mitochondrial DNA (cytochrome *b*). Phylogenetic analysis reveals potential identification of a new genus of Antillean rice rat from Guadeloupe and suggests that rice rats colonised the Lesser Antilles from South America, through at least two separate colonisation events, via over water dispersal.

5.2 Introduction

Island colonisation events provide the ideal conditions to study biological evolution, and particularly the process of adaptive radiation (Ricklefs, Bermingham, 2008). The origins of adaptive radiation can be traced to Darwin, who refers to the distribution of similar mockingbirds in the Galápagos Islands and the possibility that species, rather than remaining constant, could have altered over time. The text book example of adaptive radiation in Darwin's finches (Geospizinae) were never fully appreciated by their namesake (Sulloway, 1982). Extensive formulation of adaptive radiation theory, is however, generally attributed to a palaeontologist, G. G. Simpson (1953). Based on Simpson, Schluter (2000) defines adaptive radiation as divergence from a single ancestor, that rapidly diversifies into several species, differing in phenotypic diversity to

exploit varying environments. Consensus regarding the relative importance of variables within this definition is contentious, particularly with regards to the importance of 'rapid' speciation and the degree of diversity, leading some to suggest that the term should be rejected altogether (Olson, Arroyo-Santos, 2009). However, all modern definitions share two principal components: an increase in species numbers through common descent and adaptive diversification (Glor, in press).

That islands provide an ideal locale for the study of species radiation is widely accepted. Practical benefits of utilising islands include their small size, well-defined distinct boundaries and general tameness of island species. Islands are, relative to continents, usually much younger, and so the time since evolutionary diversification is shorter with reduced number of species and extinction events. The isolated nature of islands means that colonisation events (post any prior unification with mainland areas) are rare, thus relatively few founder lineages have the potential to radiate into novel niche environments. Oceanic islands are often volcanic, emerging from the ocean completely uninhabited and geographically isolated, restricting access and dispersal from other areas (Losos, Ricklefs, 2009). These combined elements facilitate understanding of evolutionary patterns, as consequences can often be observed or more easily interpreted, as ordered, discrete sequences of events.

Many of the classic illustrations of species radiations are found on islands. The Galápagos archipelago is perhaps the most famous, and has inspired extensive research (Parent *et al.*, 2008), including a thirty year study that highlighted continued variation in body size and beak traits of two populations of Darwin's finches (*Geospiza fortis* and *Geospiza scandens*) (Grant, Grant, 2002). The Hawaiian archipelago supports the silversword alliance (Compositae), a radiation of plants that includes 28 species from three genera that span an array of habitats including bogs and forests and range in size from small shrubs to trees (Robichaux *et al.*, 1990). The Caribbean islands are renowned for the Anoles (Anolis) radiation, in particular the Greater Antilles, where each of the islands support between 7 and 58 different species of lizard, each morphologically

distinct, exhibiting different behaviours, and utilising varying structural habitats (Losos *et al.*, 2006).

When researching island radiation events an initial question must certainly be that of colonisation, how did lineages arrive at these isolated locations? When examining islands that have never been connected to a continental landmass such as the Galápagos, a dispersal explanation is the only viable option. However, for some archipelagos the geological evidence shows ambivalent support for periods of mainland connection and disconnection. One such island complex is the Caribbean, comprising the Bahamas, Greater and Lesser Antilles (West Indies) (*Introduction chapter; Figure 1.5*). The complex geological history of the region has led to significant debate over the formation of the islands, focused on the original position of the Caribbean plate, the movement of the plates, the connectivity of landmasses and perhaps most importantly for faunal colonisations, the timing of subaerial (above water) island formation (Burke, 1988; Hedges, 2006; Iturralde-Vincent, MacPhee, 1999; Meschede, frisch, 1998) (*Introduction chapter; Section 1.6*)

This study focuses specifically on the Lesser Antillean islands, defined in the north by the Anegada Passage (the boundary with the Greater Antilles) and to the south by the continental shelf of South America (*Figure 5.1*). Initiation of the island arc system is thought to have begun during the Cretaceous, a geological period ca. 145 - 65 Myr, with the subduction of the Atlantic Plate beneath the eastern edge of the Caribbean Plate creating a proto-Antillean arc (Burke, 1988). The eastern edge of the Caribbean plate continued to move, initially forming the Aves Ridge, but with further eastward movement the Lesser Antilles (Hedges, 2006). Volcanic activity continued in the Lesser Antilles region, with a volcanic front along the *Older arc*: Grenada, Grenadines, St. Vincent, St. Lucia, Martinique, Marie-Galante, Grande Terre (eastern island of Guadeloupe), Antigua, Barbuda, St. Barthelemy, St. Martin and Anguilla at the onset of the Eocene (ca. 55 Myr).
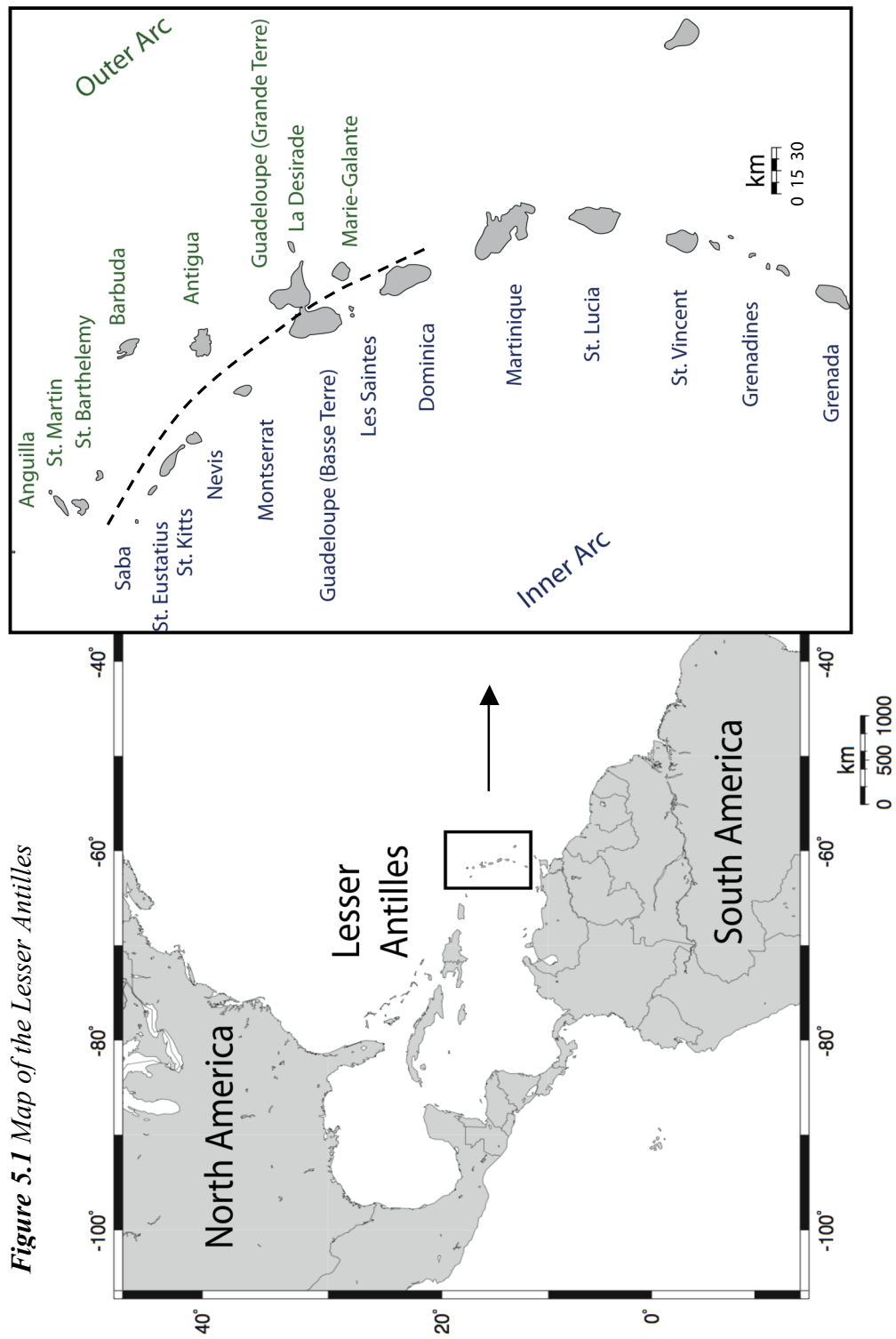
Figure 5.1 *Map of the Lesser Antilles*

Map indicating the position of the Lesser Antillean islands. The dashed line denotes the division between the outer and inner arc islands. Outer arc islands are identified in green, inner arc islands blue

After a period of activity the volcanic line ceased, but resumed after ca. 10 Myr (mid-Miocene) along the *Recent arc*: this follows the same path as the *Older arc* from Grenada to Martinique but north of Martinique diverts west along Dominica, Les Saintes, Basse Terre (western island of Guadeloupe), Montserrat, Nevis, St. Kitts, St. Eustatius, and Saba (Bouysse, Westercamp, 1990). The *Recent arc*, also termed the inner volcanic arc, remains volcanic. The older arc islands, north of Martinique (Marie-Galante and north western islands up to Anguilla) known as the outer arc remain volcanically inactive, with the volcanic rock now overlain by carbonates - they are thus sometimes referred to as the Limestone Caribbees (Paulatto *et al.*, 2010) (*Figure 5.1*).

By the Pliocene (ca. 5Myr) tectonic activity had less effect on the geographical form of the Antilles. However, the continuous cycling between glacial and interglacial periods during the Pleistocene would have led to correlated periods of xeric and mesic environments. Sediment cores from low latitude lakes in Haiti confirm that the region was significantly drier during glacial periods (Hodell *et al.*, 1991). Lake sediments were used to examine water depth through glacial and interglacial periods; the results indicated significant reduction in lake water volume during the drier glacial periods than in the wetter interglacials. Fluctuating climatic conditions would have had a substantial effect on fauna and flora, but a crucial consequence of the correlatory rise and fall in sea levels would be the exposure and submergence of island landmasses. Models incorporating ice sheets, ocean temperatures and oxygen isotope records suggest that global sea level, during peak glacial periods, was 125 +/- 12 meters below present sea level over the last million years (Bintanja *et al*., 2005). As a result of this many of the individual islands recognised today would have oscillated between submergence and emergence, as isolated islands and with unification of islands within close proximity, several times over throughout the Pleistocene. The island groups most likely to be united during periods of reduced sea level are; Anguilla /St. Martin /St. Barthelemy; St. Kitts/ Nevis /St. Eustatius; Antigua /Barbuda; Guadeloupe /La Desirade; Grenada /Grenadines (Pregill *et al.*, 1994) (*Figure 5.2*).

Figure 5.2 *Map illustrating the Lesser Antillean islands unified during lower sea levels*
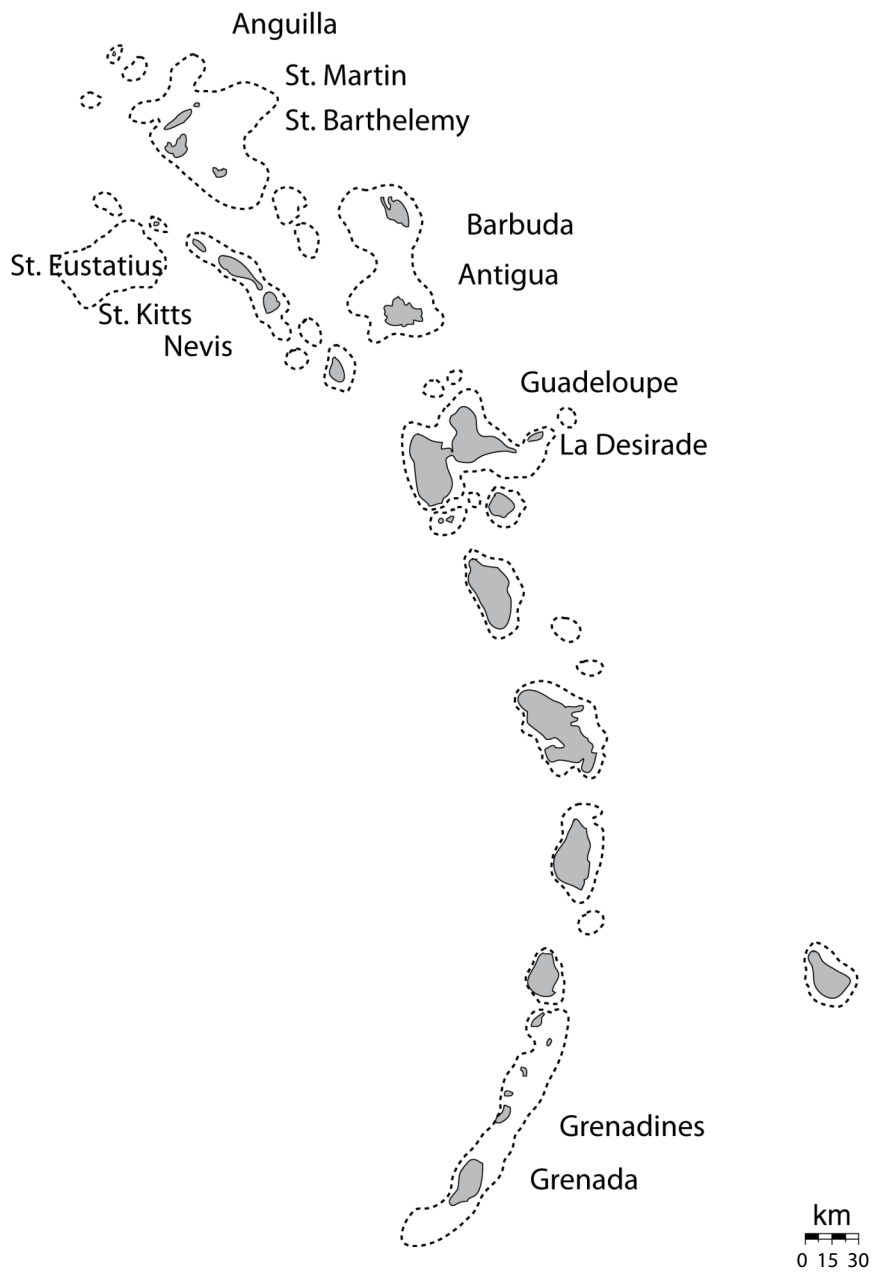
The solid lines indicate modern day sea levels; the dashed lines indicate a 200m isobath. This illustrates exposed land during glacial periods and island unification at this time. Map redrawn from (Pregill et al., 1994)

The geographical complexities of an island arc series, combined with island submergence/unification and an oscillating climate have the potential to provide a myriad of changing niche environments and shifting allopatric barriers to gene dispersal: the ideal conditions for species radiations to occur. This has been evidenced through a number of studies covering various taxonomic groups including amphibians, reptiles and birds (see below) in an attempt to understand the origin of colonising species, timing of the initial colonisation event/events and the relatedness of subsequent species.

Eleutherodactylus frogs, one of the most specious vertebrate genuses on Earth, are found throughout the New World tropics, including the Lesser Antilles. Colonisation of the Caribbean and Central America were thought to have occurred during the Cretaceous (ca. 65 Myr) via a proto-Antillean landmass (Hass, Hedges, 1991; Savage, 1982) (*Introduction chapter; Section 1.6*). Recent molecular studies, however, identified that *Eleutherodactylus* frogs colonised the Antilles during the early Cenozoic (47 - 29 Myr) via overwater dispersal from a South American lineage. Dispersal between the Caribbean islands followed, including a secondary dispersal of a Cuban lineage to the North American mainland during the Early Miocene (19 Myr) (Heinicke *et al.*, 2007). Island to mainland colonisation events, generally considered rare, have been reported for several Antillean taxa. Traditionally, Anoles were thought to have colonised the Antilles on two separate occasions, one resulting in the *roquet* group in the Southern Lesser Antilles, the other leading to all other Anoles. Molecular evidence supports the *roquet* radiation, but highlights a more complex series of dispersal and colonisation events for the remaining lineages, including two possible back colonisation events, from the Caribbean to the mainland (Nicholson *et al.*, 2005; Pinto *et al.*, 2008). Short-faced bats (Stenodermatina) colonised the Antilles via a single colonisation event in the Miocene (10.8 – 20.3 Myr) and diversified throughout the Greater and Lesser Antillean islands, with a single lineage returning to the continent leading to four current genera (Dávalos, 2007).

Ancient, stepping stone colonisation, has been reported for the pitviper (*Bothrops caribbaeus* and *Bothrops ianceolatus*) currently found on St. Lucia and Martinique. A single colonisation event is thought to have occurred from South

America to St. Luica during the late Miocene/early Pliocene (4.2 – 8.9 Myr) followed by a second dispersal from St. Lucia to Martinique (Wuster *et al.*, 2002). In contrast, a recent colonisation event from South America to the Lesser Antilles has been postulated for the boa (*Corallus enydris*) (Henderson, Hedges, 1995).

Avian fauna of the Lesser Antilles exhibit relatively little radiation compared to other archipelagos. This is possibly a consequence of the relative ease of colonisation due to the proximity of the islands to each other and the mainland, with ecological niches being swiftly filled, increasing competition and negating the possibility of species diversifying to fill them. New colonisation events can occur rapidly across the islands, both from the north (Greater Antilles) and the south (South America). Initial colonisation of contemporary birds has been estimated to date to 7.5 – 10 Myr (Ricklefs, Bermingham, 2001; 2007; 2008).

No previous research has focused on endemic non-volant land mammals in the Lesser Antilles, largely due to the absence of extant native land mammals in the region. Fossil evidence of past faunas is limited due to the volcanic nature of many of the islands and the absence of dry caves and shelters, usually rich in preserved owl pellet deposits. However, fossil evidence of reptiles, amphibians, birds and introduced mammals have been found on most of the Lesser Antillean islands (Pregill *et al.*, 1994). Fossil evidence of endemic terrestrial mammals are limited, a giant heptaxodontid rodent (*Amblyrhiza inundata*) described from Anguilla (Cope, 1869), with additional material traced to the island of St. Martin (McFarlane, MacPhee, 1989). A capybara (*Hydrochaeris gaylordi*) and fragmentary material of one or possibly two species of sloth (Megalonychidae), found in Late Cenozoic deposits on Grenada (MacPhee *et al.*, 2000). The most abundant endemic non-volant mammal recovered from the Lesser Antillean islands are from a tribe of rice rats (Oryzomyini).

As a means of better understanding how terrestrial mammals colonised the Lesser Antilles I focus on this key mammalian fauna, the extinct Antillean rice rats, (Muroidea: Sigmodontinae: Oryzomyini). The rice rat tribe is the largest within the subfamily Sigmodontinae and consists of 28 extant genera and approximately 115 species, distributed from the eastern United States and throughout Central and

South America (Weksler *et al.*, 2006). The tribe includes several endemic island species, including taxa in the Galápagos (*Aegialomys* and *Nesoryzomys*), Trinidad (*Nectomy*s) and a recently described genus from Bonaire (*Agathaeromys*) (Zijlstra *et al.*, 2010). With the exception of several Galápagos species, all other oceanic endemic island rice rat species are considered extinct (Musser, Carleton, 2005).

The Antillean rice rats, once endemic to the Lesser Antillean islands, are no exception and are thought to have become extinct within the recent historical period. Substantial samples of rice rat material have been recovered in late Holocene zooarchaeological and palaeontological contexts from nearly all of the Lesser Antilles islands (Newsom, Wing, 2004; Pregill *et al.*, 1994; Steadman *et al.*, 1984; Turvey *et al.*, in press; Wing, 2001) (*Figure 5.3*). The island of Dominica represents a notable exception as one of the largest, central islands within the chain. The absence of material is however, thought to represent a sampling artefact as opposed to genuine absence (Pregill *et al.*, 1994). Despite an abundance of subfossil material from most islands, only five rice rat species have been formally described. This 'neglect' can be attributed to the predominant recovery of rice rat remains being associated with archaeological excavations. These were primarily interested in the cultural aspects of early Amerindian settlers on the islands and any zooarcheology focused on Amerindian subsistence patterns rather than the fauna *per se* (Pregill *et al.*, 1994). Thus archaeological reports often refer to a generic rice rat. This leaves the intriguing prospect that in addition to the five described species, there are up to thirteen further possibly unique or isolated island populations of extinct Antillean rice rats that have yet to be fully investigated, making this fauna one of the largest understudied mammalian island radiations (*Table 5.1*).

Figure 5.3 *The distribution of extinct Lesser Antillean rice rats*
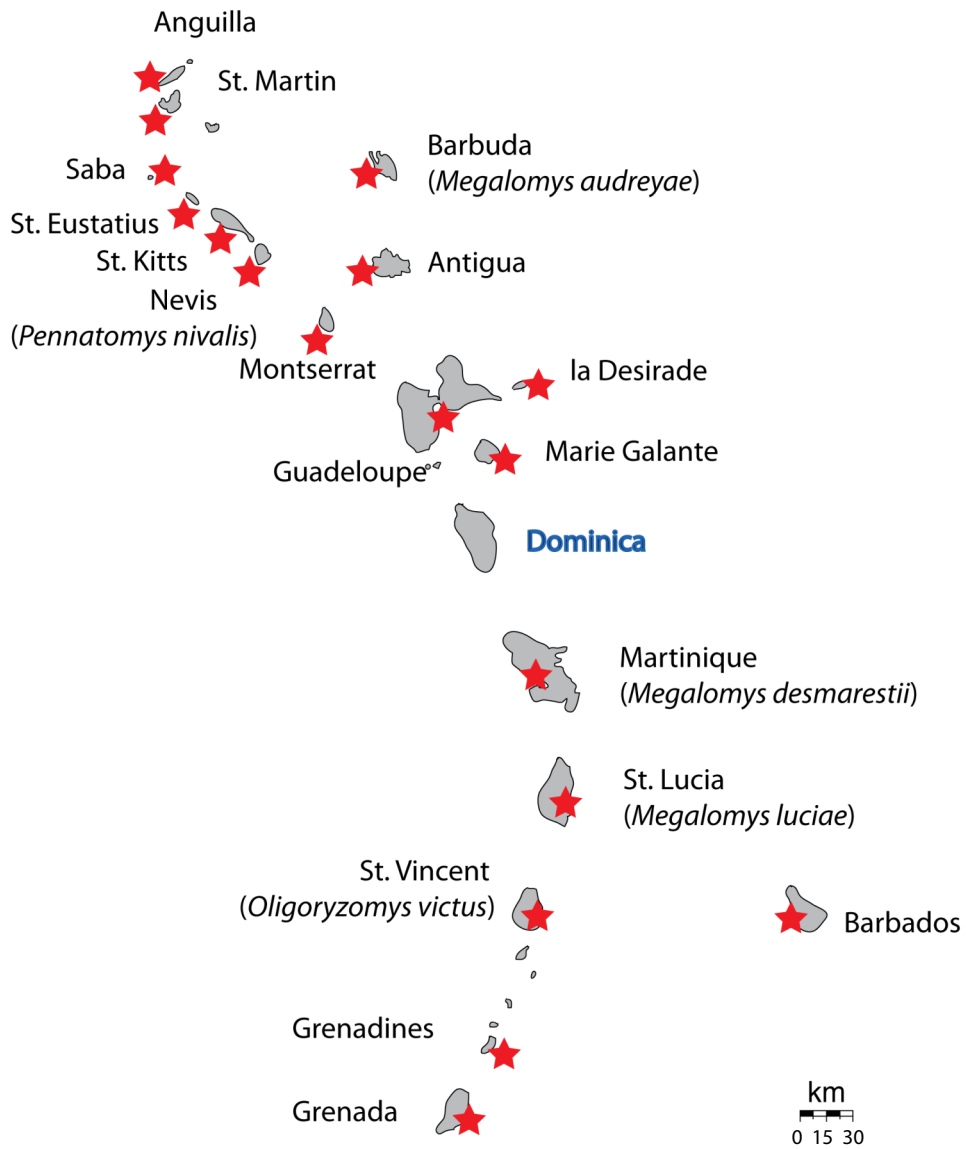
The red stars indicate islands from which extinct rice rat material has been recovered. Described species are detailed in brackets. The island of Dominica is highlighted blue

Table 5.1 *Described and undescribed rice rat material*

Species	Morph	Island	Reference
Megalomys audreyae		Barbuda	Hopwood (1926)
Megalomys desmarestii		Martinique	Fischer (1829)
Megalomys luciae		St. Lucia	Forsyth Major (1901)
Oligoryzomys victus		St. Vincent	Thomas (1898)
Pennatomys nivalis		Nevis	Turvey *et al*. (in press)
		St. Eustatius	Turvey *et al*. (in press)
		St. Kitts	Turvey *et al*. (in press)
Undescribed taxon		Anguilla	Pregill *et al*. (1994)
Undescribed taxon		Antigua	Steadman *et al*. (1984a)
Undescribed taxon		Barbados	Morgan & Woods (1986)
Undescribed taxon		Grenadines	LeFebvre (2007)
Undescribed taxon	large	Grenada	Pregill *et al*. (1994)
Undescribed taxon	small	Grenada	Pregill *et al*. (1994)
Undescribed taxon		Guadeloupe	Pregill *et al*. (1994)
Undescribed taxon		La Desirade	Pregill *et al*. (1994)
Undescribed taxon		Marie-Galante	Pregill *et al*. (1994)
Undescribed taxon	large	Montserrat	Steadman *et al*. (1984b)
Undescribed taxon	small	Montserrat	Steadman *et al*. (1984b)
Undescribed taxon		Saba	Wing (2001)
Undescribed taxon		St. Martin	Wing (2001)

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Current species identifications of rice rats from Martinique, St. Lucia and St. Vincent are based on complete specimens of animals, caught during the nineteenth century. The Barbuda rice rat was described from limited subfossil material found among cave breccia in the early 1900's (Hopwood, 1926). Rice rats from Nevis, St. Kitts and St. Eustatius have recently been described from zooarchaeological remains, with the three island populations interpreted as conspecific (Turvey *et al.*, in press).

In addition to the lack of data concerning rice rat diversity, almost nothing is known about the timing or events that led to the Antillean rice rats' colonisation of the islands. Rice rats are thought to have descended from proto-sigmodontine ancestors from North America in the late Cenozoic. The location of lineage diversification for rice rats (and all other Sigmodontine rodents), and the timing of entry for ancestral forms into South America have been controversial (Smith, Patton, 1999). Theories include:

(1) An early arrival (early to middle Miocene 15 – 24 Myr), via overwater dispersal of an ancestral form to South America (Reig, 1980).

(2) A recent arrival by overland dispersal, after the formation of the Isthmus of Panama (3.5 - 4 Myr) (Coates *et al.*, 1992). This involves either the ancestral form, followed by rapid diversification (Simpson, 1950) or,

(3) As differentiated lineages, diversification having previously occurred in North and Central America (Baskin, 1986).

Part of the controversy originates from the poor fossil record, the earliest known Sigmodontine fossils in South America are from the Montehermosan fauna of Argentina dated to 4 -5 Myr (Marshall *et al.*, 1979). The fossil record for rice rats is particularly sparse, many genera and some tribes have no recognised fossils assigned to them (Zijlstra *et al.*, 2010). The earliest fossil record is of *Carletonomys cailoi* dating to the early or middle Pleistocene from Buenos Aires, Argentina (Pardiñas, 2008). However, molecular evidence from extant rice rats estimates the diversification of the rice rat lineage to a much earlier date, between 5 – 9 Myr (Smith, Patton, 1999; Steppan *et al.*, 2004). Timing of the Lesser Antillean rice rat colonisation and the location of the closest extant relative (North or South America) therefore have the potential to address some of these key

issues. In the unlikely scenario, considering previous estimates, that diversification of the Lesser Antillean rice rats occurred ca. 65 Myr then vicariant dispersal via the proto-Antillean landmass would be inferred. Any later diversification would necessitate overwater dispersal to the Antilles. If diversification from a South American lineage of origin can be identified, the date of diversification could be utilised to set further bounds on an initial dispersal event from North America to South America. In contrast, evidence for a North American origin could indicate a greater level of diversification prior to colonisation of South America than has previously been appreciated.

In addition to the initial colonisation of the Lesser Antilles, further diversity across the islands may have been contrived by the accidental or deliberate translocation of the rice rats by Early Amerindian settlers. As previously noted the vast majority of rice rat remains have been discovered in association with archeological sites excavated to examine the settlements of the early Amerindian inhabitants of the islands. Amerindians are thought to have populated the Lesser Antilles by 4000 BP, with the earliest theory suggesting an expansion from the Greater Antilles from 7000 BP (Wilson, 1990).

Common features excavated at Amerindian sites are kitchen middens - specific areas where people disposed of food waste. Amerindians are known to have heavily exploited rice rats as a significant component of their diet, as abundant rice rat material has been recovered from kitchen midden excavations (Pregill *et al.*, 1994; Wing, 2001). The remains indicate that the animals were cooked whole, either boiled or roasted and the lack of upper jaws attached to skulls suggests that the cranium was opened in order to remove the brain. These island middens are a particularly useful tool in investigating the palaeoeconomy of early Lesser Antillean settlers, who appear to have been isolated, self-contained and mainly sedentary. Middens are therefore thought to provide a reasonably complete record of the animals consumed over a dateable period of time. An excavated midden at Indian Creek, Antigua has provided a record of food consumption for over 1,100 years (Jones, 1985). The biomass of rice rat material recovered differs between islands, with some illustrating a decrease of abundance over time (Mill Creek, Antigua; Wing *et al.*, 1968), suggesting potential over-exploitation, although other

sites, even from the same island (Indian Creek, Antigua; Jones, 1985) report abundant rice rat material throughout all excavated levels including the youngest.

Amerindians are known to have introduced captive animals to other Caribbean islands, such as the agouti (*Dasyprocta*) in the Lesser Antilles (Pregill *et al.*, 1994; Wing, 2001). As rice rats formed an extensive part of the Amerindian diet it has been suggested that it was through human translocation that the species spread across the Lesser Antillean islands. Nokkert (2002) speculates that where remains of two morphological types (small and large) have been found on the same island this could be an indication that the larger rice rats were imported there as an additional source of meat. An increase in abundance of rice rat remains from a midden in Saba (Wing, 2001) could also indicate translocation of rice rats between islands.

Depletion of rice rats through Amerindian consumption is one of the potential explanations for the extinction of the Antillean rice rats. However, abundance and continued persistence throughout excavated sites (Jones, 1985) combined with last occurrence dates for rice rats in Martinique and St. Lucia dating to 1890 – 1880 AD (Allen, 1942) suggest this is an unlikely scenario. Rice rats on Martinique certainly remained abundant into the 1650's; Allen (1942) quotes from a 1654 reference by Du Tertre that the Martinique rodent 'was commonly eaten by the people who, after singeing off the hair, exposed the body over night to the air, and then boiled it, throwing off the first water in order to get rid of the strong musky odor'. Further proposals for the extinction of the Antillean rice rats include the arrival of European colonists who conducted the large-scale forest clearance for crops such as sugarcane. They also facilitated the accidental arrival of Old World rats (*Rattus*) followed by the planned introduction of the Indian mongoose (*Herpestes javancius*) a misguided attempt to evict the black rat (Steadman *et al.*, 1984; Turvey, 2009). A combination of European peoples and exotic species arrival therefore appear the most appropriate explanations, particularly in light of the absence of competitors and native mammalian predators on the islands making them highly vulnerable to exotic species (Dávalos, Turvey, in press). What is undeniable is that if, during the nineteenth century, the Lesser Antillean islands witnessed the extinction of eighteen different

species of rice rats this represents one of the most severe mammalian extinctions of recent times.

The predominant aim of this research was to investigate this overlooked and understudied island radiation. I apply molecular analyses utilising both described and undescribed extinct rice rat material to examine species diversity across the Lesser Antillean islands and the phylogenetic relationships of Antillean rice rats. This has allowed me to establish a temporal context for rice rat colonisation of the Lesser Antilles and address broader questions with regards rodent colonisation and the Caribbean region. The main areas I propose to explore are:

(1) The extent of the Lesser Antillean rice rat radiation. Do individual island populations represent significantly diversified populations or species? If they do, is this an environmentally driven radiation or mediated through human translocation?

(2) The mode of colonisation. Did Antillean rice rats originate from the North or South of America and did they disperse via a single or multiple colonisation events?

(3) The timing of diversification events. Did ancestral rice rats disperse via a proto-Antillean landmass (ca. 65 Myr), the Isthmus of Panama (3.5 – 4 Myr) or through overwater dispersal events?

5.3 Materials and methods

5.3.1 Samples

A total of 34 samples were obtained, from eight different islands, sourced from university and museum collections (University of Southampton; Leiden University; Florida Museum of Natural History; Museum National D'Histoire Naturelle, Paris; Natural History Museum, London) (*Table 5.2*). The tissue sample from the Museum National D'Histoire Naturelle, Paris, was taken from a wild caught rice rat, brought back from St. Lucia by Bonnecour, some time previous to 1881 (Allen, 1942).

Table 5.2 *Rice rat samples*

Island	Site	Material	Estimated Age of Material	Number of Samples	Source
St. Kitts	Sugar Factory Pier	Bone	AD 700 - 1000	7	Florida Museum of Natural History
St. Eustatius	Golden Rocks	Bone	AD 1000	7	Florida Museum of Natural History
Martinique	Paquemar	Bone	AD 1000	3	Florida Museum of Natural History
Nevis	Hichmans	Bone	100 BC - AD 600	5	University of Southampton
Marie Galante	Taliseronde	Bone	AD 1000	5	Florida Museum of Natural History
St. Lucia	Wild caught	Tissue	ca. 1881	1	Museum National D'Histoire Naturelle, Paris
Guadeloupe	Anse à la Goude	Bone	AD 1000	5	Leiden University
Martinique	Wild caught	Tissue	mid nineteenth century	1	Naturural History Museum, London
				34	

The Martinique tissue sample from Natural History Museum London is specimen number NHM 50.11.30.6

5.3.2 DNA extraction

All DNA extractions were conducted in a dedicated ancient DNA laboratory, physically separated from the post- PCR laboratory. Bone samples were ground using a freezer mill (6750 SPEX CertiPrep) and liquid nitrogen and extracted using silica spin columns based on (Yang *et al.*, 1998) with the inclusion of 1M urea in the extraction buffer. Tissue and other non-bone samples were extracted using QIAamp DNA Micro kit and protocols. Mitichondrial DNA (MtDNA) was amplified using overlapping fragments spanning 460 base pairs of the cytochrome b region. A total of nine primer pairs were utilised in this study, (*Appendix K*), each primer pair amplifying short (140 – 180 base pairs) overlapping fragments. PCR reactions were performed using a final concentration of 1 x PCR buffer, 0.2μM of each primer, 250μM dNTPs, 2mM $MgSO_4$, 1mg/ml BSA, 1 Unit Platinum Taq DNA polymerase high fidelity, purified water, and 2μl of DNA extract in a 25μl mix. PCR conditions were 5 min at 95°C, followed by 55 cycles of 1 min at 92°C, 1 min at between 48°C and 52°C (dependent on primer pair specifications), 1 min at 68°C, and with a final extension of 5 min at 68°C.

Amplicons were purified using Exonuclease I and Shrimp Alkaline Phosphatase. Sequencing reactions were performed by Macrogen (Korea) using a high throughput genetic analysis sequencer, ABI3730XL. Sequencing chromatograms were assembled and analysed using Sequencher 4.0 analysis software (Gene Codes Corporation). Standard ancient DNA protocols (Gilbert *et al.*, 2005) were followed throughout these extraction procedures to prevent contamination, with repeated PCR amplification and sequencing of fragments to ensure DNA authenticity and the absence of miscoding lesions.

5.3.3 Phylogenetic analyses

DNA sequences obtained from this study were aligned by eye in Se-AL 2.0 (Rambaut 2002). To investigate the relationship of the Lesser Antillean rice rats and extant rice rats, cytochrome b sequence data of extant species were sourced from Genbank. Data were selected with preference for published sequence data but attempted to cover all genera within the rice rat tribe. No data were available for *Amphinectomys*, *Lundomys*, *Microakodontomys* and *Mindomys* genera, but the 23 remaining extant genera are represented in this analysis (*Table 5.3*).

Phylogenetic relationships were estimated using Maximum Likelihood (ML) and Bayesian methods. The DNA substitution model selected with ModelTest3.7 (Posada, Crandall, 1998) under Akaike Information Criterion (AIC) was General Time Reversible (GTR) with proportion of invariable sites (I) set to 0.4883 and gamma distribution (G) shape parameter 0.7175. Maximum Likelihood trees were generated in PAUP* (Swofford, 2000) using a full heuristic search, based on branch swapping with tree-bisection-reconnection (TBR). Bayesian trees were constructed using MrBayes 3.1 (Ronquist, Huelsenbeck, 2003), implementing nucleotide substitution model General Time Reversible with gamma distribution (GTR+G), four chains (three heated one cold) were run for one million generations with a burn-in period of 1000 trees. Nodal support was assessed using approximate posterior probabilities performed in MrBayes. A pinyon mouse (*Peromyscus truei*) sequence sourced from Genbank (Accession number AF108703.1) was utilised as the outgroup.

Divergence estimates were calculated in BEAST (Drummond, Rambaut, 2007) using the GTR substitution model, with gamma plus invariant site estimates (as specified by Modeltest), Yule prior parameter (suitable for individuals that represent different species), a strict molecular clock and a mutation rate of 4% per million years, a moderate rodent mutation rate for cytochrome *b* (Hadly *et al.*, 2004; Irwin *et al.*, 1991). Further analysis explored estimated mutation rates when divergence dates were fixed to correlate with either an ancient land connection ca. 65 Myr, the closing of the Isthmus of Panama ca. 3.75 Myr or Amerindian translocation ca. 7 Kyr. In all analyses chain length was set to 10, 000 000, data collected every 1000 with a burn-in of 10,000.

Pairwise estimates of sequence divergence with Kimura 2-parameter (K2P) (Kimura 1980) distances were calculated in PAUP*.

Table 5.3 *Extant rice rat sequences obtained from Genbank*

Genus	Species	Accession Number	Location	Reference
Aegialomys	xanthaeolus	EU579479	Peru: Arequipa	Hanson & Bradley (Unpublished)
Ereoryzomys	polius	EU579483	Peru: Amazonas Balsas	Hanson & Bradley (Unpublished)
Euryoryzomys	emmonsae	AF251525	Brazil: Para	Patton et al. (2000)
Euryoryzomys	emmonsae	AF251526	Brazil: Para	Patton et al. (2000)
Handleyomys	alfaroi	EU579489	Nicaragua: Matagalpa	Hanson & Bradley (Unpublished)
Handleyomys	rostratus	EU579492	Mexico: Tamaulipas	Hanson & Bradley (Unpublished)
Holochilus	brasiliensis	EU579495	Paraguay: Neembucu	Hanson & Bradley (Unpublished)
Holochilus	chacarius	DQ227455	Paraguay: Presidente Hayes	Amman et al. (Unpublished)
Holochilus	sciureus	AF108697	Brazil: Amazonas	Smith & Patton (1999)
Hylaemys	acritus	AY940625	Bolivia	Emmons & Patton (2005)
Hylaemys	laticeps	AF251522	Brazil: Bahia	Patton et al. (2000)
Hylaemys	laticeps	AF251521	Brazil: Para	Patton et al. (2000)
Hylaemys	megacephalus	AY275124	N/A	D'Elia (2003)
Hylaemys	megacephalus	AF251519	Brazil: Para	Patton et al. (2000)
Hylaemys	yunganus	AF251520	Brazil: Amazonas	Patton et al. (2000)
Melanomys	caliginosus	EU340020	Ecuador: Esmeraldes	Hanson & Bradley (Unpublished)
Microryzomys	minutus	U58387	Peru	Patton & da Silva (1995)
Neacomys	spinosus	AF108701	Peru: Amazonas	Smith & Patton (1999)
Nectomys	apicalis	U03539	Peru: Cusco	Smith & Patton (1999)
Nectomys	squamipes	AF181283	Brazil: Fazenda da Mata	Bonvicino & Moreira (2001)
Nectomys	squamipes	AY041195	Bolivia: Beni	Rinehart et al. (Unpublished)
Nephelomys	albigularis	DQ224407	N/A	Amman et al. (Unpublished)
Nesoryzomys	fernandinae	AF108700	Ecuador: Galapagos Archipelago	Smith & Patton (1999)
Oecomys	bicolor	AF108699	Peru: Amazonas	Smith & Patton (1999)
Oecomys	bicolor	U58382	Brazil	Patton & da Silva (1995)
Oecomys	superans	AY275123	Peru: Amazonas	D'Elia (2003)
Oecomys	superans	U58385	Brazil	Patton & da Silva (1995)
Oecomys	roberti	U58384	Brazil	Patton & da Silva (1995)
Oecomys	trinitatus	U58390	Brazil	Patton & da Silva (1995)
Oligoryzomys	andinus	AY452200	N/A	Palma et al. (2005)
Oligoryzomys	chacoensis	AY275706	Chile: Magallanes	Palma et al. (2005)
Oligoryzomys	fornesi	AY452199	Paraguay: Misiones	Palma et al. (2005)
Oligoryzomys	fulvescens	DQ227457	USA: Texas, Cameron County	Milazzo et al. (2006)
Oligoryzomys	longicaudatus	AY452198	Chile: Magallanes	Palma et al. (2005)
Oligoryzomys	longicaudatus	AY452197	Chile: Magallanes	Palma et al. (2005)
Oligoryzomys	microtis	U58381	Brazil	Patton & da Silva (1995)
Oligoryzomys	microtis	AY439000	Bolivia: Santa Cruz	Carroll et al. (2005)
Oligoryzomys	nigripes	EU258551	Paraguay: Neembucu	Hanson (Unpublished)
Oryzomys	macconnelli	AF251528	Brazil: Amazonas	Patton et al. (2000)
Oryzomys	macconnelli	AF251527	Brazil: Amazonas	Patton et al. (2000)
Oryzomys	russatus	AF181272	Brazil: Guapimirim	Bonvicino & Moreira (2001)
Oryzomys	russatus	AF181271	Brazil: Fazenda Aldeia	Bonvicino & Moreira (2001)
Oryzomys	nitidus	U58383	Brazil: Acre	Patton & da Silva (1995)
Oryzomys	nitidus	AF251529	Peru: Cuzcu	Patton et al. (2000)
Oryzomys	lamia	AF181273	Brazil: Fazenda Fiandeira	Bonvicino & Moreira (2001)
Oryzomys	couesi	DQ370034	USA: Texas, Cameron County	Milazzo et al. (2006)
Oryzomys	couesi	DQ185386	Mexico: Oaxaca, Las Minas	Milazzo et al. (2006)
Oryzomys	palustris	DQ370033	USA: Texas, Brazoria County	Milazzo et al. (2006)
Oryzomys	palustris	DQ370032	USA: Oklahoma, Okmulgee	Milazzo et al. (2006)
Oryzomys	balneator	EU258534	Ecuador: Napo	Hanson (Unpublished)
Oryzomys	azuerensis	EU074669	Panama: Veraguas	Hanson et al. (Unpublished)
Oryzomys	azuerensis	EU074668	Panama: Veraguas	Hanson et al. (Unpublished)
Oryzomys	mexicanus	EU074655	Mexico: Jalisco	Hanson et al. (Unpublished)
Oryzomys	mexicanus	EU074654	Mexico: Michoacan	Hanson et al. (Unpublished)
Oryzomys	texensis	FJ974124	USA: Louisiana, Cameron Parish	Hanson et al. (2010)
Oryzomys	texensis	FJ974123	USA: Louisiana, Cameron Parish	Hanson et al. (2010)
Pseudoryzomys	simplex	EU579517	Paraguay: Neembucu	Hanson & Bradley (Unpublish
Scolomys	juruaense	AF108696	Brazil: Amazonas	Smith & Patton (1999)
Scolomys	juruaense	U58386	Brazil: Amazonas	Patton & da Silva (1995)
Scolomys	melanops	AF527419	Peru: Loreto	Gomez-Laverde et al. (2004)
Sigmodontomys	alfari	EU340016	Ecuador: Esmeraldes	Hanson & Bradley (2008)
Sooretamys	angouya	EU579512	Paraguay: Neembucu	Hanson & Bradley (Unpublish
Transandinomys	talamancae	EU579515	Panama: Bocas del Toro	Hanson & Bradley (Unpublish
Zygodontomys	brevicauda	EU579521	Venezuela: Sucre	Hanson & Bradley (Unpublish
Zygodontomys	cherriei	U579520	Venezuela: Zulia	Hanson & Bradley (Unpublish
Peromyscus	truei	AF108703	USA: California	Smith & Patton (1999)
Peromyscus	schmidlyi	AY322516	Mexico: Durango	Bradley et al. (2004)

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5.4 Results

5.4.1 DNA sequence data

From the 34 rice rats sampled, 7 successfully yielded mtDNA, two samples from the island of St. Kitts, and one from Nevis, St. Eustatius, Martinique, St. Lucia and Guadeloupe.

The low success rate for this study can be attributed to a number of factors. The predominant concern - also conversely an area of interest - from the commencement of this project, was the potential for the material to be highly degraded through their exposure to a humid subtropical climate (*Introduction chapter; Section 1.8.2*). The concern was obviously founded, but not insurmountable as attested by the (limited) success achieved from bone samples and positive results from tissue samples. The two tissue samples (Martinique and St. Lucia) were obtained from deceased animals that had been caught in the wild, thus DNA - post death - was not exposed to the same tropical environment, both samples successfully yielded mtDNA.

A factor that was not fully appreciated until the preliminary stages of this study were the specious nature of the rice rat tribe and the extent of molecular diversity between species. This posed a significant problem during primer design for several reasons. Firstly, no extant species could be readily dismissed as the position and relatedness of the Lesser Antillean rice rats within the rice rat tribe was unclear. Secondly, cytochrome b sequence data from extant species (obtained from Genbank) were highly diverse. Conserved regions of mtDNA across species that would normally be targeted as primer sites - in order to maximise the likelihood of binding with unknown target DNA - were therefore scarce. An alternative solution would have been to utilise a conserved region of mtDNA such as 16S rDNA. This was rejected, as it was unlikely to achieve sufficient resolution for the species level differences of interest to this study. The identification of workable primer pairs therefore went through successive periods of trial and design. The unknown diversity within the Antillean rice rats further impeded the trial process. Potential island specific differences meant that every primer pair needed to be tested on material from each of the islands before they could be

definitively rejected. These factors, combined with the degraded nature of the DNA, made identification of workable primer pairs and the successful amplification of mtDNA acutely difficult.

The accumulation of workable primer pairs and reliable mtDNA sequence data was therefore a lengthy process, testing approximately 20 different primer pairs and performing nearly 500 PCR reactions. Likely due to the degraded nature of the DNA, sequencing chromatograms were often of poor quality and therefore rejected. All sequences utilised in this study were verified through repeated amplification and sequencing of each of the targeted DNA fragments, at least twice. When the sequence obtained was partially unclear or ambiguous further replications were carried out. If ambiguity persisted but remained restricted to less than 5 ambiguous bases, the bases were recorded following the International Union of Pure and Applied Chemistry (IUPAC) ambiguity code, any greater ambiguity and the entire fragment was rejected. The data reported here, although limited, has therefore been rigorously monitored for authenticity, successfully sequenced fragments for each of the samples are detailed in *Figure 5.4*

Figure 5.4 *Schematic of primer pairs and successfully sequenced fragments*
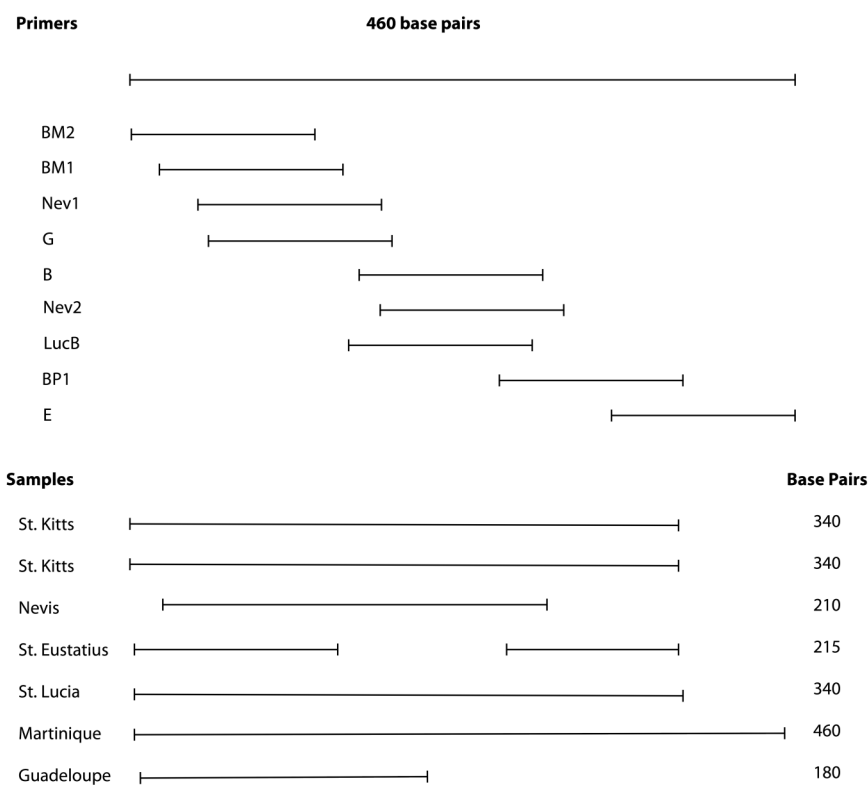
5.4.2 Phylogenetic analyses

Maximum Likelihood and Bayesian analyses generated congruent phylogenetic trees, thus only the Bayesian tree is shown, with both the approximated Bayesian posterior probabilities values indicated (*Figure 5.5*). Samples sequenced from this study are highlighted in colour. The samples from St. Kitts, Nevis, St. Eustatius, Martinique and St. Lucia form a discrete Lesser Antillean clade. The sample from Guadeloupe represents a separate lineage.

The level of genetic diversity between islands samples were estimated through corrected pairwise sequence divergence using Kimura 2-parameter distances (*Table 5.4*).

Table 5.4 *Percentage sequence divergence between samples*

	St. Kitts	St. Kitts	Nevis	St. Eustatius	St. Lucia	Martinique	Guadeloupe
St. Kitts							
St. Kitts	1.22						
Nevis	4.5	5.54					
St. Eustatius	7.95	6.93	9.73				
St. Lucia	14.51	13.24	16.02	11.7			
Martinique	15.4	13.98	15.9	14.4	3.65		
Guadeloupe	25.65	26.2	30.15	31.99	18.9	19.31	

Percentage sequence divergence for extant conspecific rice rats utilised in this analysis ranged from 0.22% – 5.4% and congeneric sequence divergence ranged from 0.88% – 20.88%.

The timing of diversification between the Lesser Antillean islands samples and from mainland extant species were estimated through dating of the most recent common ancestor between individual island groups and the mainland sister taxa (*Table 5.5*). When divergence dates were fixed, the estimates for mutation rate are shown in *Table 5.6*.

Table 5.5 *Time since the most recent common ancestor of Lesser Antillean and mainland rice rat species*

Most Recent Common Ancestor Between	Divergence Dates (Myr)		
	95% HPD lower	95% HPD upper	Mean
Main Lesser Antillean clade & *Cerradomys* sp.	4.4	9.59	6.95
Main Lesser Antillean clade	2.66	6.12	4.28
St. Lucia & Martinique	0.34	1.36	0.79
St. Eustatius, Nevis & St. Kitts	1.01	2.72	1.78
Nevis & St. Kitts	0.45	1.84	1.09

Table 5.6 *Fixed divergence dates and the resultant estimates for mutation rates*

Most Recent Common Ancestor Between	Divergence Date	Mutation Rate (% per million years)		
		95% HPD lower	95% HPD upper	Mean
Main Lesser Antillean clade & *Cerradomys* sp.	65 Myr	0.271	0.484	0.373
Main Lesser Antillean clade & *Cerradomys* sp.	3.75 Myr	4.74	8.48	6.44
Main Lesser Antillean clade	3.75 Myr	3.08	6.29	4.66
St. Lucia & Martinique	7 Kyr	221	932	564

Figure 5.5 *Phylogeny for the rice rats*
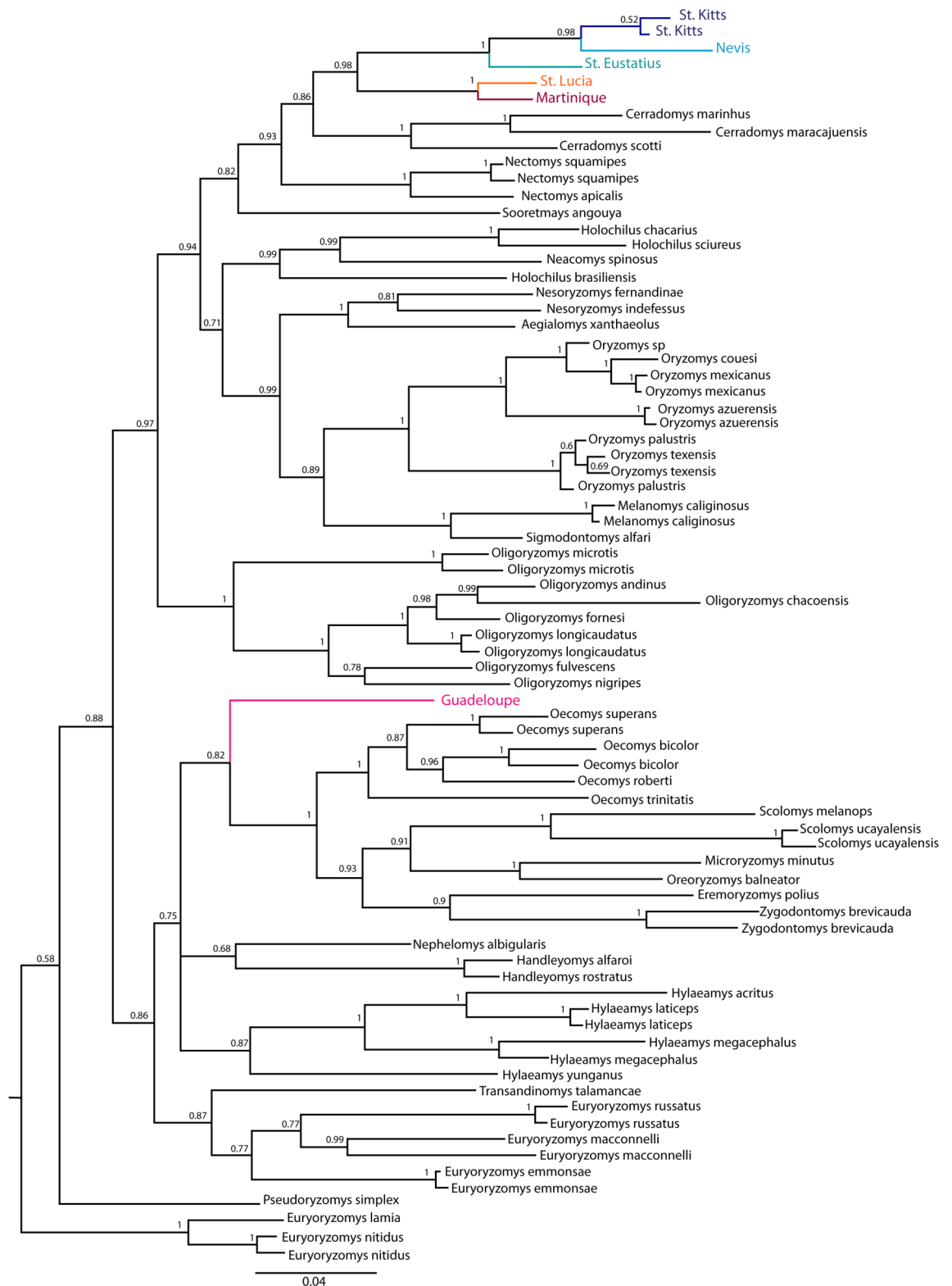
Bayesian tree constructed in MrBayes with GTR for 1,000,000 generations. Nodal support is shown through approximate Bayesian probabilities above the line. Permoyscus truei used as an outgroup (not shown). Extinct Lesser Antillean samples are highlighted in colour.

5.5 Discussion

The phylogenetic relationships recovered from this research have provided new insights both within the extinct Antillean rice rats and concerning their association with extant mainland species. The phylogenetic structure (*Figure 5.5*) indicates a main Antillean rice rat clade that includes samples from St. Kitts, Nevis, St. Eustatius, Martinique and St. Lucia. The association between Antillean rice rat taxa within this clade is robustly supported by the Bayesian posterior probabilities. The close association between samples from St. Kitts, Nevis and St. Eustatius are in accordance with the close proximity of the islands and recent morphological findings. While the morphological findings indicated minor body size differences between the three islands, and were interpreted to represent a single species (Turvey *et al.*, in press), the molecular diversity reported here is relatively high, 4.5% – 9.73% (*Table 5.4*) when compared to sequence divergence of conspecific extant rice rats used in this study (0.22% - 5.4%).

Data from previous studies have reported mean conspecific sequence divergence using 11 species of sigmodontine rodents sampled from two or more geographic localities to be 3.7% (range 0.4% – 9.7%) (Smith, Patton, 1999). A further study reported sequence divergence within two Oryzomyini species to range between <1% and 3% (Myers *et al.*, 1995). The percentage sequence divergences reported here, between the islands of St. Kitts, Nevis and St. Eustatius are therefore among the highest reported for conspecific sigmodontine rodents. This could indicate that the three populations represent individual species. I propose that the data recovered are too limited to fully assess whether this is an accurate reflection of species level divergence, but certainly suggest the need for further molecular sampling.

Rice rats from neighbouring islands Martinique and St. Lucia are also recovered as sister taxa. Sequence divergence was relatively low (3.65%), lower than the divergence reported for the Nevis, St. Eustatius and St. Kitts group, but higher than that reported for the two St. Kitts samples (1.22%). Congeneric extant rice rats utilised in this study ranged in sequence divergence from 0.88% to 20.88%; the Martinique and St. Lucia samples therefore fall well within this range. The

morphological distinction combined with molecular divergence (albeit low) can therefore be used to interpret the samples as separate species. The sister relationship of rice rats from Martinique and St. Lucia are also consistent with previous morphological reports (Turvey *et al.*, in press; Weksler, 2006). The association of the Martinique and St. Lucia material and samples from St. Kitts, Nevis and St. Eustatius into a discrete Antillean rice rat clade had not previously been recovered. The only prior study to incorporate the Nevis rice rat in a wider rice rat phylogeny (Turvey *et al.*, in press) reported low statistical support for its placement within the same broad clade as rice rats from Martinique and St. Lucia, but not as sister taxa. The strong support recovered for the discrete clade observed in this analysis therefore constitutes a more robust placement for these taxa. The monophyletic clade incorporating (St. Kitts, Nevis, St. Eustatius, St. Lucia and Martinique) indicates that all of these species can be interpreted as arising from the same lineage, and are thus most parsimoniously explained as the result of a single colonisation event.

The rice rat from Guadeloupe, however, provides a more complex scenario. It does not fall within the main Lesser Antillean rice rat clade defined above; indeed its relatedness to other Antillean rice rats is remote, with sequence divergence ranging from 18.9% to 31.99%. While this is highly interesting, the position of this taxa receives very low statistical support and its precise placement is therefore dubious. Even so, PCR amplification of the extracted DNA provided clear sequencing chromatograms, 30% of the 180 base pairs used in this analysis incorporated overlapping primer pairs with all data replicated twice from separate PCR reactions. An alignment search using BLAST 2.2.24 (Zhang *et al.*, 2000) reported the top 40 most significant alignments as extant rice rat species and the sample falls inside the rice rat phylogeny generated. I therefore conclude that the sample is unlikely to represent contaminant mtDNA. Further, despite the inability to place the sample with any confidence at a particular branching node the level of sequence divergences from other Antillean rice rats are high. I therefore propose that although its position within the phylogeny as a whole is unclear, it is unlikely that it forms part of the main Antillean rice rat clade.

This is informative, particularly as the Guadeloupe sample represents one of the undescribed island populations. The high sequence divergence between this specimen and other described species of Antillean rice rat is highly indicative that the Guadeloupe sample represents a new genus and species. Obviously, the limited data reported here is insufficient, however with additional molecular and morphological data I suggest that this will confirm a new genus and species. In the scarce literature relating to a Guadeloupe rice rat, remains have been reported as 'un-described species B', a large rice rat, potentially the same species as found on Montserrat, Barbuda, Antigua and Marie Galante (Pregill *et al.*, 1988). Unfortunately, with the exception of Marie Galante, I was unable to obtain samples from the above islands and failed to obtain sequenceable aDNA from the Marie Galante specimens. Therefore, I am currently unable to verify or dispute these associations; only noting at present that it appears markedly distinct from all other Antillean rice rats analysed in this study.

Aside from it's potential identification as a new genus, if the position of the Guadeloupe sample is accepted as distinct from the main Antillean rice rat clade this indicates multiple rice rat colonisation events in the Lesser Antilles. If a single colonisation event had occurred, with a subsequent radiation of species from this lone ancestral lineage, a single, reciprocally monophyletic clade would have been recovered. The phylogenetic pattern observed from this analysis indicates that the closest mainland common ancestor of the Guadeloupe rice rat lineage was different from that of the main Antillean clade, thus implying paraphyletic diversification. This may have occurred simultaneously or at different time points, but the lack of shared ancestral lineage suggests that the colonisation of the Lesser Antilles did not transpire from a single colonisation event.

Poor support and thus an unstable position for the Guadeloupe sample within the rice rat phylogeny invalidates any attempt to date the time of the split between this sample and mainland taxa. However, the height of the Guadeloupe branch is comparable to the height of the main Antillean clade, potentially suggestive of a similar time period of divergence. The ancestral node between the main Antillean rice rat clade and mainland species has significant statistical support and can

therefore be dated with greater confidence. The estimated divergence date for the main Antillean rice rat clade and the closest extant mainland species (*Cerradomys*) was estimated to between 4.4 and 9.59 Myr (95% HPD) with a mean value of 6.95 Myr (*Table 5.5*). The date is consistent with previous estimates for diversification of the rice rat lineage (5 – 9 Myr) (Smith, Patton, 1999; Steppan *et al.*, 2004). It is also consistent with the assertion that rice rats did not colonise the Antilles via a proto-Antillean landmass (65 Myr), and thus necessitates an over-water dispersal event. That a mean mutation rate of 0.373 % per million years (*Table 5.6*) - an order of magnitude slower than a typical mutation rate for cytochrome *b* - would be required for the divergence of the main Antillean rice rat clade and *Cerradomys* to be fixed to 65 Myr, further supports over-water dispersal as the most likely mode of colonisation.

Timing of the initial colonisation of the Lesser Antilles and subsequent radiation are estimated to have occurred between 2.66 and 6.12 Myr (95% HPD) with a mean value of 4.28 Myr. Both the diversification from a mainland species and the colonisation of the Antilles are therefore estimated to have occurred prior to the closing of the Isthmus of Panama (3.5 – 4 Myr) (Coates *et al.*, 1992). While the lower bound for time since the Lesser Antillean clade ancestor (2.66 Myr), could date the radiation post this event, the lineage split is not basal, thus rice rat diversification likely occurred before the Panamanian land bridge linked North and South America. Similarly, when the divergence date for the most recent common ancestor of 1) the main Antillean rice rat clade and *Cerradomys* and 2) the main Antillean rice rat clade were fixed to 3.75 Myr (closing of the Isthmus of Panama) the estimated mutation rates required are 1) 6.44 % per million years and 2) 4.66% per million years (*Table 5.6*). While the mutation rates appear faster than expected they are not excessively so, thus divergence could have occurred around the time of the formation of the land bridge. However, as previously noted with regards to the direct estimates of divergence dating, these splits are not basal, thus rice rat divergence likely occurred prior to this event.

In addition, the mainland lineages most closely related to the Lesser Antillean rice rats are the genera *Cerradomys* and *Nectomys,* both of which are distributed in South America. It can therefore be inferred that the Lesser Antillean rice rats are

descended from a South American lineage and that diversification likely occurred prior to the closing of the Isthmus of Panama.

The timing of diversification events between the Lesser Antillean island lineages between 0.34 and 1.36 Myr (95% HPD) with a mean value of 0.79 Myr and between 1.01 and 2.72 Myr (95% HPD) with a mean value of 1.78 Myr, is also highly informative as these events significantly pre-date the mid-Holocene arrival of the earliest Amerindian colonists (Wilson, 1990). The island species estimated to have diverged in the most recent past are from the islands of St. Lucia and Martinique. When the divergence date for this event is fixed to the earliest estimate of Amerindian colonisation (7000 BP), the mean mutation rate required is estimated at 564 % per million years (*Table 5.6*). This rate is two orders of magnitude faster than a typical cytochrome *b* mutation rate. Therefore Amerindian translocation of rice rats between islands can also be rejected as a component of the rice rat radiation across the Lesser Antillean island chain.

The position of the main Antillean rice rat clade yields further information in regard to the current distribution and characteristics of the closest living relatives of the extinct rice rats. The wider Antillean clade illustrates that the main group of extinct rice rats are most closely related to extant mainland species of the genus *Cerradomys* and *Nectomys*. *Cerradomys* species are distributed in South America from northeastern Brazil to southeastern Bolivia and northwestern Paraguay (Percequillo *et al.*, 2008) (*Figure 5.6*). Species within the genus *Nectomys* are also distributed across South America, from the north of Columbia across the northern and eastern edge of South America, throughout the Amazon basin and west into Bolivia and Peru, remaining east of the Andes (Hershkovitz, 1944; Weksler, 2006) (*Figure 5.7*).

That both genera are distributed in South America and particularly along the northeastern coast represents further evidence of overwater dispersal. Both genera can be found within the Guiana Shield region (located between the Orinoco and Amazon Rivers) an area highlighted as highly significant for overwater dispersal. Drainage from major rivers in these regions would flow directly into the clockwise current driven by the Coriolis force capable of transporting flotsam

from this region and depositing it in the region of the Lesser Antilles (Hedges, 2001; Hedges, 1996). The importance of the Coriolis force in driving currents and directionality of flotsam in the region has been criticised, predominantly on the grounds that this is an oversimplification that fails to take into account additional factors that induce or alter frictional forces. The added complications of forces such as wind directionality would result in more random dispersal of flotsam that could take weeks or months to reach the Antilles, greatly reducing the likelihood of faunal survival (MacPhee, Iturralde-Vincent, 2005). However, as previously noted (Hedges, 2006), while overwater dispersal would be unsuccessful on the vast majority of occasions, only a single successful voyage is required for colonisation to occur.

Figure 5.6 *Cerradomys distribution*   **Figure 5.7** *Nectomys distribution*

Distributions for both mainland genera are within the Guiana Shield depicted as a dashed square between the Orinoco and Amazon Rivers. Cerradomys are distributed across the region highlighted red, Nectomys are distributed across the region highlighted green

An additional point of interest associated with the overwater dispersal capabilities of Antillean rice rats relates to the close association with *Nectomys*. Extant species of rice rat are commonly ground dwelling or semi-arboreal, however, species of *Nectomys* are among the handful of semi-aquatic rice rats. These species, along with *Lundomys*, *Sigmodontomys alfari*, *Pseudoryzomys*, *Amphinectomys and*

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Oryzomys palustris are commonly associated with an aquatic habitat and exhibit morphological adaptations for a semi-aquatic lifestyle including well-developed interdigital webbing and natatory fringes (stiff hairs on the hindfeet) (Weksler, 2006). *Nectomys* species are considered among the most highly adapted of the rice rats for semi-aquatic life, exclusively found near bodies of water such as swamps, lakes and wooded streams (Hershkovitz, 1944). Santori *et al.* (2008) examined rice rat locomotory specialisations for swimming and a semi-aquatic existence. Focal species of investigation were the terrestrial rice rat (*Cerradomys*) and the semi-aquatic rice rat (*Nectomys*) (found in this study to be most closely related to the extinct Antillean rice rats). Results illustrated that both were capable of bipedal paddling, but that *Nectomys* were faster, possessed greater hydrodynamic posture and exhibited reduced water absorption of the fur. They were also unique in their ability to float effortlessly on the water surface for long periods of time (Santori *et al.*, 2008).The natural habitat and semi-aquatic nature of *Nectomys*, one of the Lesser Antillean rice rats closest relatives, imply a common association with rivers and river material, both now and in the past. This increases the likelihood that they could have been washed out to sea on a raft of vegetation, and survive a lengthy overwater journey.

The constraints of working with highly degraded material have limited the amount of data compiled in this study, but succeeded in demonstrating the potential for tropical material to yield results. To summarise the information collated, I refer to the three focal areas of interest as stated at the end of the introduction, section 5.1:

(1) The extent of the Lesser Antillean rice rat radiation. From the molecular diversity reported here, there is potential identification of a new genus of Antillean rice rat from Guadeloupe, and suggestion that the *Pennatomys* genus from Nevis may constitute two further species from St. Eustatius and St. Kitts. These aspects require further investigation. However, it is clear from this study that the Antillean rice rats represent significantly diverse populations. The timing of the diversification between these lineages could not have been mediated through human translocation, thus the Antillean rice rats represent a major environmentally driven evolutionary radiation.

(2) The mode of colonisation. The Antillean rice rats appear to have colonised the Lesser Antilles through at least two separate colonisation events, though

additional molecular data would be required to make a robust case for the Guadeloupe colonisation. Support for the main Antillean clade with *Cerradomys* and *Nectomys* species as their closest living relatives is strong and provides positive indication that colonisation of the Lesser Antilles - in this case - originated from South America.

(3) The timing of mainland to island diversification events. The divergence date for the main Antillean rice rat clade and the closest mainland species (*Cerradomys*) is estimated to between 4.4 and 9.59 Myr (95% HPD) with a mean value of 6.95 Myr. This is significantly later than any proposed land connection between the Antilles and North or South America. Further, when lineage divergence is fixed to the time of the last land connection (65 Myr) the estimated mean mutation rate is 0.373 %/Myr, a value significantly slower than any previously reported for the cytochrome *b* region. A vicariant land dispersal event is therefore rejected. It also highlights that ancestral rice rats may have colonised South America prior to the formation of the Isthmus of Panama, as diversification of the Antillean clade from a South American, non-basal lineage, likely occurred prior to this event.

Rice rat colonisation of the Lesser Antilles is therefore consistent with overwater waif dispersal. A theory corroborated by close association with semi-aquatic species and distribution of sister taxa across the Guiana Shield region, an area where river mouths are ideally placed to feed into the clockwise current that flows towards the Lesser Antillean islands.

5.6 References

Allen GM (1942) *Extinct and vanishing mammals of the Western Hemisphere with the marine species of all the oceans* American Committee for International Wild Life Protection, Washington DC.

Baskin JA (1986) The late Miocene radiation of Neotropical sigmodontine rodents in North America. *Rocky Mountain Geology* **24**, 287-303.

Bintanja R, Wal RSWvd, Oerlemans J (2005) Modelled atmospheric temperatures and global sea levels over the past million years. *Nature* **437**, 125 - 128.

Bonvicino CR, Moreira MA (2001) Molecular phylogeny of the genus Oryzomys (Rodentia: Sigmodontinae) based on cytochrome b DNA sequences. *Molecular Phylogenetics and Evolution* **18**, 282-292

Bouysse P, Westercamp D (1990) Subduction of Atlantic aseismic ridges and Late Cenozoic evolution of the Lesser Antilles island arc. *Tectonophysics* **175**, 349-355, 357-380.

Bradley RD, Carroll DS, Haynie ML, Martinez RM, Hamilton MJ, Kilpatrick CW (2004) A new species of Peromyscus from western Mexico. *Journal of Mammalogy* **85**, 1184-1193

Burke K (1988) Tectonic Evolution of the Caribbean. *Annual Review of Earth and Planetary Sciences* **16**, 201-230.

Carroll DS, Mills JN, Montgomery JM, Bausch DG, Blair PJ, Olson JG, Rogers DS, Salazar M, Ksiazek TG (2005) Hantavirus pulmonary syndrome in Central Bolivia: relationships between reservoir hosts, habitats, and viral genotypes. *American Journal of Tropical Medicine and Hygiene* **72**, 42-46

Coates AG, Jackson JBC, Collins LS*, et al.* (1992) Closure of the Isthmus of Panama: The near-shore marine record of Costa Rica and western Panama. *Geological Society of America Bulletin* **104**, 814-828.

Cope ED (1869) Exhibition of bones and teeth of a large rodent from the cave deposits of Anguilla, one of hte West India islands. *Proceedings Academy of Natural Sciences, Philadelphia*, 313.

Dávalos LM (2007) Short-Faced Bats (Phyllostomidae: Stenodermatina): A Caribbean Radiation of Strict Frugivores. *Journal of Biogeography* **34**, 364-375.

Dávalos LM, Turvey ST (in press) West Indian mammals: the old, the new, and the recently extinct. In: *Historical Biogeography of the Neotropics* (ed. Patterson BD). Chicago University Press, Chicago.

Drummond A, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**, 214.

Fischer, JB (1829) Synopsis Mammalium. J. G. Cottae, Stuttgardtiae

Forsyth Major CI (1901) The musk-rat of Santa Lucia (Antilles). *Annals and Magazine of Natural History* Series 7, **7**, 204-206

Gilbert MTP, Bandelt H-Jr, Hofreiter M, Barnes I (2005) Assessing ancient DNA studies. *Trends in Ecology & Evolution* **20**, 541-544.

Glor RE (in press) Phylogenetic Insights on Adaptive Radiation. *Annual Review of Ecology, Evolution and Systematics*.

Gomez-Laverde M, Anderson RP, Garcia LF (2004) Integrated systematic reevaluation of the Amazonian genus Scolomys (Rodentia: Sigmodontinae) *Zeitschrift für Säugetierkunde* **69**, 119-139

Grant PR, Grant BR (2002) Unpredictable Evolution in a 30-Year Study of Darwin's Finches. *Science* **296**, 707-711.

Hadly EA, Ramakrishnan U, Chan YL*, et al.* (2004) Genetic Response to Climatic Change: Insights from Ancient DNA and Phylochronology. *PLoS Biology* **2**.

Hanson, J.D (unpublished) Molecular phylogenetics of the Oryzomyini using multiple genes

Hanson JD, Bradley RD (2008) Molecular diversity within Melanomys caliginosus (Rodentia: Oryzomyini): Evidence for multiple species. *Occasional papers - Museum, Texas Tech University* **275**, 1-11

Hanson JD, Bradley RD (unpublished) Molecular phylogenetics of Oryzomyini: does a multi-gene approach help resolve a systematic conundrum?

Hanson JD, Fulhorst CF, Bradley RD (unpublished) Molecular Divergence of Oryzomys palustris and O. couesi: a Taxonomic Reassessment.

Hanson JD, Indorf JL, Swier VJ Bradley RD (2010) Molecular divergence within the Oryzomys palustris complex: evidence for multiple species *Journal of Mammalogy* **91**, 336-347.

Hass CA, Hedges SB (1991) Albumin evolution in West Indian frogs of the genus *Eleutherodactylus* (Leptodactylidae): Caribbean biogeography and a

calibration of the albumin immunological clock. *Journal of Zoology* **225**, 413-426.

Hedges BS (2001) Biogeography of the West Indies: An Overview. In: *Biogeography of the West Indies Patterns and Perspectives* (eds. Woods CA, Sergile FE), pp. 15 - 35. CRC Press, New York.

Hedges SB (1996) Historical Biogeography of West Indian Vertebrates *Annual Review of Ecology and Systematics* **27**, 163-196.

Hedges SB (2006) Paleogeography of the Antilles and Origin of West Indian Terrestrial Vertebrates *Annals of the Missouri Botanical Garden* **93**, 231-244.

Heinicke MP, Duellman WE, Hedges SB (2007) Major Caribbean and Central American frog faunas originated by ancient oceanic dispersal. *Proceedings of the National Academy of Sciences* **104**, 10092-10097.

Henderson RW, Hedges SB (1995) Origin of West Indian Populations of the Geographically Widespread Boa Corallus enydris Inferred from Mitochondrial DNA Sequences. *Molecular Phylogenetics and Evolution* **4**, 88-92.

Hershkovitz P (1944) Systematic review of the Neotropical water rats of the genus *Nectomys* (Cricetinae). *Miscellaneous Publications, Museum of Zoology, University of Michigan* 1-110

Hodell DA, Curtis JH, Jones GA*, et al.* (1991) Reconstruction of Caribbean climate change over the past 10,500 years. *Nature* **352**, 790.

Hopwood AT (1926) A fossil rice-rat from the Pleistocene of Barbuda. *Annals and Magazine of Natural History* Series 9, **17**, 328-330.

Irwin D, Kocher T, Wilson A (1991) Evolution of the cytochrome *b* gene of mammals. *Journal of Molecular Evolution* **32**, 128-144.

Iturralde-Vincent MA, MacPhee RDE (1999) Paleogeography of he Caribbean Region: Implications for Cenozoic Biogeography. *Bulletin of the American Museum of Natural History* **238**, 1-95.

Jones AR (1985) Dietary Change and Human Population at Indian Creek, Antigua. *American Antiquity* **50**, 518-536.

LeFebvre MJ (2007) Zooarchaeological analysis of prehistoric vertebrate exploitation at the Grand Bay Site, Carriacou, West Indies. *Coral Reefs* **26**: 931–944.

Losos JB, Glor RE, Kolbe JJ, Nicholson K (2006) Adaptation, Speciation, and Convergence: A hierarchial analysis of adaptive radiation in Caribbean Anolis Lizards. *Annals of the Missouri Botanical Garden* **93**, 24-33.

Losos JB, Ricklefs RE (2009) Adaptation and diversification on islands. *Nature* **457**, 830-836.

MacPhee RDE, Iturralde-Vincent MA (2005) The Interpretation of Caribbean Paleogeography: Reply to Hedges. *Insular Vertebrate Evolution*, 175 - 184.

MacPhee RDE, Singer R, Diamond M (2000) Late Cenozoic Land Mammals from Grenada, Lesser Antilles Island-Arc. *American Museum Novitates* **3302**, 1-20.

Marshall LG, Butler RF, Drake RE, Curtis GH, Tedford RH (1979) Calibration of the Great American Interchange. *Science* **204**, 272-279.

McFarlane DA, MacPhee RDE (1989) Amblyrhiza and the Quaternary Bone Caves of Anguilla, British West Indies. *Cave Science* **16**.

Milazzo ML, Cajimat MN, Hanson JD, Bradley RD, Quintana M, Sherman C, Velasquez RT, Fulhorst CF (2006) Catacamas virus, a hantaviral species naturally associated with Oryzomys couesi (Coues' Oryzomys) in Hondouras. *American Journal of Tropical Medicine and Hygiene* **75**, 1003-1010

Meschede M, frisch W (1998) A plate-tectonic model for the Mesozoic and Early Cenozoic history of the Caribbean plate. *Tectonophysics* **296**, 269-291.

Morgan GS, Woods CA (1986) Extinction and the zoogeography of West Indian land mammals. *Biological Journal of the Linnean Society* **28**: 167–203.

Musser GG, Carleton MC (2005) Superfamily Muroidea. In: *Mammal Species of the World. A Taxonomic and Geographic Reference* (eds. Wilson DE, Reeder DM), pp. 963 - 966. Johns Hopkins University Press, Baltimore.

Myers P, Lundrigan B, Tucker PK (1995) Molecular Phylogenetics of Oryzomyine Rodents: The Genus Oligoryzomys. *Molecular Phylogenetics and Evolution* **4**, 372-382.

Newsom LA, Wing ES (2004) *On land and sea. Native American uses of biological resources in the West Indies* University of Alabama Press.

Nicholson KE, Glor RE, Kolbe JJ*, et al.* (2005) Mainland colonization by island lizards. *Journal of Biogeography* **32**, 929-938.

Nokkert M (2002) The animal remians from the Hichmans' Site Complex (GE-5 and GE-9), Nevis (Lesser Antilles) Remains from the 2002 excavation, pp. 1-33.

Olson ME, Arroyo-Santos A (2009) Thinking in continua: beyond the "adaptive radiation" metaphor. *BioEssays* **31**, 1337-1346.

Palma RE, Rivera-Milla E, Salazar-Bravo J, Torres-Perez F, Pardinas UFJ, Marquet PA, Spotorno AE, Meynard AP, Yates TL (2005) Phylogeography of Oligoryzomys longicaudatus (Rodentia: Sigmodontinae) in temperate South America. *Journal of Mammalogy* **86**, 191-200.

Pardiñas UFJ (2008) A New Genus of Oryzomyine Rodent (Cricetidae: Sigmodontinae) from the Pleistocene of Argentina. *Journal of Mammalogy* **89**, 1270-1278.

Parent CE, Caccone A, Petren K (2008) Colonization and diversification of Galápagos terrestrial fauna: a phylogenetic and biogeographical synthesis. *Philosophical Transactions of the Royal Society B: Biological Sciences* **363**, 3347-3361.

Patton JL, da Silva MNF (1995) A review of the spiny mouse genus Scolomys (Rodentia: Muridae: Sigmodontinae) with the description of a new species from the western Amazon of Brazil. *Proceedings of the Biological Society of Washington* **108**, 319-337

Patton JL, da Silva MNF, Malcolm JR (2000) Mammals of the Rio Jurua and the evolutionary and ecological diversification of Amazonia. *Bulletin of the American Museum of Natural History* **244** 1-306

Paulatto M, Minshull TA, Baptie B*, et al.* (2010) Upper crustal structure of an active volcano from refraction/reflection tomography, Montserrat, Lesser Antilles. *Geophysical Journal International* **180**, 685-696.

Percequillo AR, Hingst-Zaher E, Bonvivino CR (2008) Systematic Review of Genus *Cerradomys* Weksler, Percequillo and Voss, 2006 (Rodentia: Cricetidae: Sigmodontinae: Oryzomyini), with Description of Two New Species From Eastern Brazil. *American Museum Novitates* **3622**, 1-46.

Pinto G, Mahler DL, Harmon LJ, Losos JB (2008) Testing the island effect in adaptive radiation: rates and patterns of morphological diversification in

Caribbean and mainland Anolis lizards. *Proceedings of the Royal Society B: Biological Sciences* **275**, 2749-2757.

Posada D, Crandall K (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817-818.

Pregill GK, Steadman DW, Olson SL, Grady FV (1988) Late Holocene Fossil Vertebrates from Burma Quarry, Antigua, Lesser Antilles. *Smithsonian Contributions to Zoology* **463**.

Pregill GK, Steadman DW, Watters DR (1994) Late Quaternary vertebrate faunas of the Lesser Antilles: historical components of Caribbean biogeography. *Bulletin of the Carnegie Museum of Natural History* **30**, 1-51.

Rambaut, A (2002) Sequnce Alignment Editor. Ver. 2.0. Available via http://evolve.zoo.ox.ac/software/se-AL/main.html.

Reig OA (1980) A new fossil genus of South American cricetid rodents allied to Wiedomys, with an assessment of the Sigmodontinae. *Journal of Zoology* **192**, 257-281.

Ricklefs R, Bermingham E (2001) Nonequilibrium Diversity Dynamics of the Lesser Antillean Avifauna. *Science* **294**, 1522-1524.

Ricklefs R, Bermingham E (2007) The Causes of Evolutionary Radiations in Archipelagoes: Passerine Birds in the Lesser Antilles. *The American Naturalist* **169**, 285-297.

Ricklefs R, Bermingham E (2008) The West Indies as a laboratory of biogeography and evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences* **363**, 2393-2413.

Robichaux RH, Carr GD, Liebman M, Pearcy RW (1990) Adaptive Radiation of the Hawaiian Silversword Alliance (Compositae- Madiinae): Ecological, Morphological, and Physiological Diversity. *Annals of the Missouri Botanical Garden* **77**, 64-72.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572-1574.

Santori RT, Vieira MV, Rocha-Barbosa O, Magnan-Neto JA, Gobbi N (2008) Water Absorption of the Fur and Swimming Behavior of Semiaquatic and Terrestrial Oryzomine Rodents. *Journal of Mammalogy* **89**, 1152-1161.

Savage JM (1982) The Enigma of the Central American Herpetofauna: Dispersals or Vicariance? *Annals of the Missouri Botanical Garden* **69**, 464-547.

Schluter D (2000) *The Ecology of Adaptive Radiation* Oxford University Press, Oxford.

Simpson GG (1950) History of the fauna of Latin America. *American Scientist* **38**, 361-389.

Simpson GG (1953) *The Major Features of Evolution* Columbia University Press.

Smith MF, Patton JL (1999) Phylogenetic Relationships and the Radiation of Sigmodontine Rodents in South America: Evidence from Cytochrome *b*. *Journal of Mammalian Evolution* **6**, 89-128.

Steadman DW, Pregill GK, Olson SL (1984a) Fossil Vertebrates from Antigua, Lesser Antilles: Evidence for Late Holocene Human-Caused Extinctions in the West Indies. *Proceedings of the National Academy of Sciences of the United States of America* **81**, 4448-4451.

Steadman DW, Watters DR, Reitz EJ, Pregill GK (1984b) Vertebrates from archaeological sites on Montserrat, West Indies. *Annals of the Carnegie Museum* **53**, 1–29.

Steppan SJ, Adkins RM, Anderson J (2004) Phylogeny and Divergence-Date Estimates of Rapid Radiations in Muroid Rodents Based on Multiple Nuclear Genes. *Systematic Biology* **53**, 533-553.

Sulloway FJ (1982) Darwin and his finches: The evolution of a legend. *Journal of the History of Biology* **15**, 1-53.

Swofford DL (2000) Phylogenetic analysis using parsimony (* and other methods),. Sinaur Associates, Sunderland, Massachusetts.

Thomas O (1898) On indigenous Muridea in the /west Indies; with the description of a new Mexican Oryzomys. *Annals and Magazine of Natural History* **7**, 176-180

Turvey ST (2009) *Holocne Extinctions* Oxford University Press.

Turvey ST, Weksler M, Morris EL, Nokkert M (in press) Taxonomy, phylogeny, and diversity of the extinct Lesser Antillean rice rats (Sigmodontinae: Oryzomyini), with description of a new genus and species. *Zoological Journal of the Linnean Society*.

Weksler M (2006) Phylogenetic Relationships of Oryzomine Rodents (Muroidea: Sigmodontinae): Separate and Combined Analyses of Morphological and Molecular Data. *Bulletin of the American Museum of Natural History* **296**, 1-149.

Weksler M, Percequillo AR, Voss RS (2006) Ten New Genera of Oryzomyine Rodents (Cricetidae: Sigmodontinae). *American Museum Novitates*, 1-29.

Wilson S (1990) Peopling the Antilles. Columbus encountered a rich and complex culture, a "singularly friendly" people whose ancestors had colonized the Caribbean over the course of six millennia. *Archaeology* **43**, 52-57.

Wing ES (2001) The sustainability of resources used by Native Americans on four Caribbean islands. *International Journal of Osteoarchaeology* **11**, 112-126.

Wing ES, Hoffman CA, Ray CE (1968) Vertebrate remains from Indian sites on Antigua, West Indies. *Caribbean Journal of Science* **8**, 123-129.

Wuster W, Thorpe RS, Salomao MG*, et al.* (2002) Origin and phylogenetic position of the Lesser Antillean species of Bothrops (Serpentes, Viperidae): biogeographical and medical implications. *Bulletin of The Natural History Museum. Zoology Series* **68**, 101-106.

Yang DY, Eng B, Waye JS, Dudar JC, Saunders SR (1998) Improved DNA extraction from ancient bones using silica-based spin columns. *American Journal of Physical Anthropology* **105**, 539-543.

Zhang Z, Schwartz S, Wagner L, Miller W (2000) A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology* **7**, 203-214.

Zijlstra JS, Madern PA, van den Hoek Ostende LW (2010) New genus and two new species of Pleistocene oryzomyines (Cricetidae: Sigmodontinae) from Bonaire, Netherlands Antilles. *Journal of Mammalogy* **91**, 860-873.

Chapter 6: Discussion

6.1 Brief summary of the data chapters

Chapter 2

An investigation of arctic collared lemming populations, an Arctic species in northern Europe through a phylochronological approach spanning a period prior to the LGM until the Younger Dryas. Analyses identified three discrete clades that suggest two population extinction events that were followed by recolonisation. Turnover events occurred during MIS 2.

Chapter 3

Examined European water vole, with particular reference to populations in the UK, from the Pleistocene through to the Holocene including modern populations. Analyses revealed that the UK had been colonised on two separate occasions, the first colonisers were replaced by a second wave during MIS 1 in England, this second wave, however, did not replace the population in Scotland.

Chapter 4

Explored the history of the Hispaniolan hutia to assess whether the current population structure reflected the ancient divisions of the island. The results highlighted the presence of three distinct hutia lineages that reflected the ancient subdivisions within the island.

Chapter 5

Focused on a group of extinct rodents, the Antillean rice rats of the Caribbean. Analyses revealed that rice rats colonised the region via over water dispersal from the South American mainland, potentially on two separate occasions.

6.2 Thesis aims

Here I refer to the overall aims of this thesis as established at the end of the introduction, section *1.12*

6.2.1 (Aim A) To assess the utility of rodent ancient DNA analyses

A1: *Determine if sample masses are sufficient to permit the generation of authentic DNA sequence data.*

Sample materials utilised in this study mainly consisted of rodent mandibles, but included a limited number of soft tissue scrapings. The mass of individual bone materials ranged from approximately 50 mg – 800 mg. The first ancient DNA studies used approximately 2 - 4 g of bone for extraction purposes (Hagelberg, Clegg, 1991), a current standard would be 150 mg (Prost *et al.*, 2010) . Despite the small mass of individual sample materials, authentic DNA sequence data were obtained from each of the 4 species groups examined in the course of this thesis.

A2: *Determine if the amount of material required for extraction and purification procedures can be further reduced to permit additional analyses such as radiocarbon dating.*

Reducing the amount of bone required whilst maintaining successful generation of authentic DNA sequence data was particularly important to the arctic collared lemming study. One of the aims of that study was to determine whether a single arctic collared lemming mandible – mass ranged from 50 mg to 180 mg - could be utilised to generate both authentic DNA sequence data and a reliable AMS date. The minimum requirement of bone material for AMS dating – dependent on the age and preservation of the sample – is approximately 100 mg. Throughout the study I reduced the mass of bone powder required in the extraction process to a minimum of 11 mg and successfully sequenced and AMS dated 8 individual arctic collared lemming mandibles. The DNA extraction procedure for these samples utilised between 11 mg and 25 mg of bone powder, the AMS dating utilised the remaining material that ranged in mass between 100 mg and 160 mg. Therefore European Late Pleistocene material were successfully sequenced using an initial bone mass that was two orders of magnitude less than earlier studies, and one order less than many current studies.

A3: *Determine if it is possible to extract authentic DNA sequence data from small sample masses where the material may be highly degraded material - both through time and warm climate.*

The oldest individual sample – an arctic collared lemming mandible from Belgium - to generate DNA sequence data and to be successfully AMS dated, returned a median calibrated age of 30, 917 yr BP, a period prior to the LGM. The DNA extraction procedure for this sample utilised 21 mg of bone powder, the AMS dating utilised the remaining 125 mg of bone. This period - just prior to the LGM - represents the extent of the planned timeframe under investigation and is regarded as a successful example of amplification of temporally degraded material.

In general, frozen and permafrost deposits have proved advantageous for long-term DNA survival and retrieval in aDNA studies (Chapter 1 section 1.8.2, aDNA degradation). In order to address areas of interest to this thesis, samples could not be sourced from such ideal environments. The more recent, Caribbean samples – the hutia – were reasonably well preserved and yielded a 66% success rate. The archaeological – Antillean rice rat samples – resulted in a much lower success rate, with only 21 % of samples analysed successfully generating aDNA sequence data. All aDNA work adhered to standards for authenticating 'genuine aDNA' (Gilbert *et al.*, 2005). In the rice rat study, with prior knowledge that the material had an increased propensity to be highly degraded, additional replications of extractions, amplifications and sequencing were routinely carried out to further ensure validity. Having undertaken these precautionary procedures I determine that authentic aDNA can be obtained from a tropical climate. The caveat is that success rate depreciates more rapidly with time, likely as a consequence of the increased rate of DNA degradation. In addition, this study highlights that well-preserved material can also be obtained from cave sites, with samples obtained from the Belgian site – TAW – exhibiting excellent DNA preservation (88% success rate).

6.2.2 (Aim B) To identify any consistent patterns across rodent species

B1: *In response to climate change*
Over a comparable depth of time, that encompassed periods of fluctuating climate, both the arctic collared lemming and the water vole studies found evidence of population turnover events. The arctic collared lemming data

indicated two extinction events followed by recolonisation during MIS 2, the more recent, ca. 20 – 21 Kyr and the earliest ca. 20 – 28 Kyr. The first turnover event could have been associated with warming climate during GI 3, but the latter, however, occurred during a period of global climatic stability, when conditions appear favourable – cold - for arctic collared lemming. In comparison, water vole exhibited remarkable stability for a temperate species during glacial conditions. ADNA analyses revealed that Pleistocene and Younger Dryas samples consistently formed an association with modern Scottish samples, indicating genetic contribution and continuity over an environmentally unstable period. A contrasting situation was revealed regarding the English water vole population, where a second wave of colonisers – most likely of Eastern European origin – completely replaced the first population ca. 8 – 12 Kyr. This occurs at approximately the time of the transition from the Younger Dryas cold phase into the current warm phase. The effects of climatic and environmental change are therefore inconsistent not only across rodent species, but within the same species. I therefore report an absence of repeated response patterns to recorded changes in climatic conditions.

B2: *With reference to genetic diversity*

It has previously been reported that aDNA analyses consistently detect a pattern of diversity depletion, past populations exhibited far greater genetic diversity than current populations (Hofreiter, Barnes, 2010). Results from the arctic collared lemming analyses indicated a 'typical' response pattern, genetic diversity decreased from the Pleistocene into the Holocene. In contrast, water vole diversity appears to be maintained across the Pleistocene Holocene transition. The disparity in genetic diversity is interesting particularly as both species operate under a similar cyclic population structure. A purely speculative explanation would be that closer proximity to a source population could have facilitated maintenance of water vole diversity, but this would require further investigation.

6.2.3 (Aim C) To review the merits of working with rodent aDNA

C1: *With regards to otherwise obscured or irretrievable data*

Analyses of current, water vole genetic data, detected a division between water vole populations in England and those in Scotland, but were unable to determine historical events that had resulted in this divide. The application of an aDNA approach permitted a direct observation into the genetic diversity of past populations in the UK. The results established that the current population in Scotland represent an initial wave of colonisers found throughout England, until ca. 12 Kyr, that were replaced by a second wave of colonisers ca. 8 – 12 Kyr. This second wave represent the genetic lineage currently found in England.

Genetic data of the extinct Antillean rice rats would obviously have been unobtainable without the application of an aDNA approach. With regards to taxonomy and phylogenetics much can be gleaned from morphology. The aDNA however, provided the means to estimate timing of colonisation events and through this, established that rice rats colonised the Lesser Antilles via over water dispersal with subsequent 'natural' radiation across islands as opposed to mediated human translocation.

C2: Concerning broader topics of general interest/concern

The association between population turnover in the arctic collared lemming and a hiatus in the mammoth record that occurred temporally and spatially localised, provided an unexpected and interesting correlation. It does not predict why these events occurred simultaneously, however it does suggest that an associated event would need to be relevant both to the large and small mammal fauna, rejecting a size-biased correlate such as human hunting.

The inclusion of aDNA in the water vole study, not only highlighted past events in the UK, but also provided a direct indication of past European genetic structure. The resultant phylogeny indicated pre-LGM samples from across Europe were grouped within modern haplogroup clades. This illustrated that current populations are not exclusively derived from founder individuals, restricted to southern peninsula during glacial periods. Thus supporting an increasing body of evidence that suggests postglacial recolonisation of Europe involved a more complex chain of events than those proposed by the southern refugia model.

The identification of three evolutionary distinct lineages of Hispaniolan hutia was of interest to establish whether ancient geological events and biogeographical distinctions affect rodent evolutionary processes. The Hispaniolan hutia is one of only two remaining endemic land mammals in the region and is currently regarded as endangered by the International Union for Conservation of Nature and Natural Resources (IUCN). This study could therefore provide relevant information for targeted conservation in the region, regarding estimations of effective population size and diversity. Population viability can provide pivotal information through the identification of regions that are of highest concern and regions where there is a greater probability of successful recovery.

Analyses of the Antillean rice rats not only estimated the timing of colonisation events within the island chain but also provided evidence concerning Sigmodontine expansion into South America. Debate regarding the timing and order of diversification has centred on the sparse fossil record and lack of consensus over whether the formation of the Panamanian land bridge was a necessary precursor for rodent entrance to South America. Timing of the ancestral diversification of the Antillean rice rats pre-dates formation of the Isthmus of Panama and is more closely associated with South American lineages. This therefore suggests that expansion of the Sigmodontine rodents was not restricted through the absence of a land bridge.

6.3 Summary

To do the evolution of rodents would be a task of immense proportions likely to ramble on, beyond the scope of this thesis. Instead, I have focused on a subsection of species, permitting exploration of a wide geographical range. The inclusion of ancient DNA has enhanced this work through temporal depth into past histories and events. Over all this has provided a wider fuller appreciation of the complexities of evolutionary processes within rodents. As study organisms they have been shown to elicit quite individual response patterns, but I hope to have illustrated their capacity to examine issues of broader concern.

6.4 Future work

There are several avenues of work undertaken in this thesis that have potential for expansion. The water vole study would be ideal to extend and encompass a more thorough sampling effort across Europe. Finer sampling would I suspect uncover the path of the Eastern European expansion and I would be particularly interested to sample Germany and Belgium to a greater extent, to explore the possibility that they are suture zones between the first and second colonisers. Other areas where I would have liked to have obtained more extensive geographical sampling, include the northern palaeo-island of Hispaniola and the remaining Lesser Antillean islands.

In a broader outlook, the work undertaken in this thesis has been based on single genes, encoded on mtDNA. While I believe this has been suitable to address the questions under investigation, technology has progressed and high throughput and third generation sequencing hold potential to generate an increasing amount of data. The inclusion of nuclear markers would therefore be advantageous to these studies through finer resolution of population structure with the potential to reveal further patterns unidentified by mtDNA.

6.5 References

Gilbert MTP, Bandelt H-Jr, Hofreiter M, Barnes I (2005) Assessing ancient DNA studies. *Trends in Ecology & Evolution* **20**, 541-544.

Hagelberg E, Clegg JB (1991) Isolation and Characterization of DNA from Archaeological Bone. *Proceedings: Biological Sciences* **244**, 45-50.

Hofreiter M, Barnes I (2010) Diversity lost: are all Holarctic large mammal species just relict populations? *BMC Biology* **8**, 46.

Prost S, Smirnov N, Fedorov VB*, et al.* (2010) Influence of Climate Warming on Arctic Mammals? New Insights from Ancient DNA Studies of the Collared Lemming Dicrostonyx torquatus. *PLoS one* **5**, e10447.

Appendices

Chapter 2

Appendix A: *Table of calibrated dates reported in the chapter that are referenced as uncalibrated dates in the literature*

Page Number	Calibrated Date (Kyr BP)	Uncalibrated Date (Kyr BP)	Reference
53	24	20	Stuart *et al*. 2004
53	13.7	12	Stuart *et al*. 2004
53	12	10.7	Stuart *et al*. 2004
53	7.9	7	Stuart *et al*. 2004
53	39 - 38	34 - 33	Stuart 2005
53	17	15	Grayson 2007
53	23	19	Grayson 2007
53	21	18	Stuart 2005
54	13.7	12	Stuart 2005
54	11.5	10	Lõugas *et al.* 2002
54	6.6	5.7	Veltre *et al*. 2008
54	4	3.7	Vartanyan *et al*. 2008

Appendix B: Table of arctic collared lemmings sampled

My Identification	Country	Site	Layer	Square	Spit	Cyt b base pairs
S148	Belgium	TAW	4	K4	5a	780
S147	Belgium	TAW	4	K4	5b	780
S036	Belgium	TAW	12	M3	1	780
S037	Belgium	TAW	12	M3	1	780
S038	Belgium	TAW	12	M3	1	780
S039	Belgium	TAW	12	M3	1	780
S040	Belgium	TAW	12	M3	1	780
S049	Belgium	TAW	12	M3	1	780
S051	Belgium	TAW	12	M3	1	780
S126	Belgium	TAW	12	M3	2	780
S127	Belgium	TAW	12	M3	2	780
S128	Belgium	TAW	12	M3	2	780
S041	Belgium	TAW	12	M3	9	780
S043	Belgium	TAW	12	M3	9	780
S046	Belgium	TAW	12	M3	9	780
S047	Belgium	TAW	12	M3	9	780
S048	Belgium	TAW	12	M3	9	780
S052	Belgium	TAW	12	M4	5	780
S053	Belgium	TAW	12	M4	5	780
S056	Belgium	TAW	12	M4	5	780
S058	Belgium	TAW	12	M4	5	780
S061	Belgium	TAW	12	M4	5	780
S054	Belgium	TAW	12	M4	5	780
S055	Belgium	TAW	12	M4	5	780
S057	Belgium	TAW	12	M4	5	780
S062	Belgium	TAW	12	M4	5	780
S064	Belgium	TAW	12	M4	5	780
S065	Belgium	TAW	12	M4	5	780
S066	Belgium	TAW	12	M4	5	780
S129	Belgium	TAW	12	M4	6	780
S130	Belgium	TAW	12	M4	6	780
S131	Belgium	TAW	12	M4	6	780
S072	Belgium	TAW	12	M4	6	780
S078	Belgium	TAW	12	M4	6	780
S079	Belgium	TAW	12	M4	6	780
S070	Belgium	TAW	12	M4	6	780
S071	Belgium	TAW	12	M4	6	780
S073	Belgium	TAW	12	M4	6	780
S074	Belgium	TAW	12	M4	6	780
S080	Belgium	TAW	12	M4	6	780
S081	Belgium	TAW	12	M4	6	780
S085	Belgium	TAW	12	M4	6	780
S086	Belgium	TAW	12	M4	6	780
S087	Belgium	TAW	12	M4	6	780
S135	Belgium	TAW	14	O7	16	780
S136	Belgium	TAW	14	O7	16	780
S133	Belgium	TAW	14	K6	9	780
S134	Belgium	TAW	14	K6	9	780
S132	Belgium	TAW	14	K7	9	780
S144	Belgium	TAW	16	M7	19	780
S145	Belgium	TAW	16	M7	16	780
S146	Belgium	TAW	16	M7	16	780
S149	UK	Bridged Pot cave				780
S150	UK	Bridged Pot cave				780
S151	UK	Bridged Pot cave				780
S152	UK	Bridged Pot cave				780
S153	UK	Bridged Pot cave				780
S050	Belgium	TAW	12	M3	1	<200
S045	Belgium	TAW	12	M3	9	<200
S137	Belgium	TAW	14	O7	16	<200
S143	Belgium	TAW	16	M6	1	<200
S140	Belgium	TAW	15B	M9	8	0
S141	Belgium	TAW	15B	M9	8	0
S142	Belgium	TAW	15B	M9	8	0
S138	Belgium	TAW	15B	M9	10	0

Appendix C: *Profile of samples from Layer 4, redrawn from Miller et al. (2005)*

x = 10,37, y = 4,08

-3,29

-3,52

4

-3,57

1

5

2

-3,86

Sample
1 layer 4 K4 spit 5a
2 layer 4 K4 spit 5b

6

-4,09

7

-4,40

8

-4,72

9

-5,01

10

-5,32

11

-5,71

0   50 cm
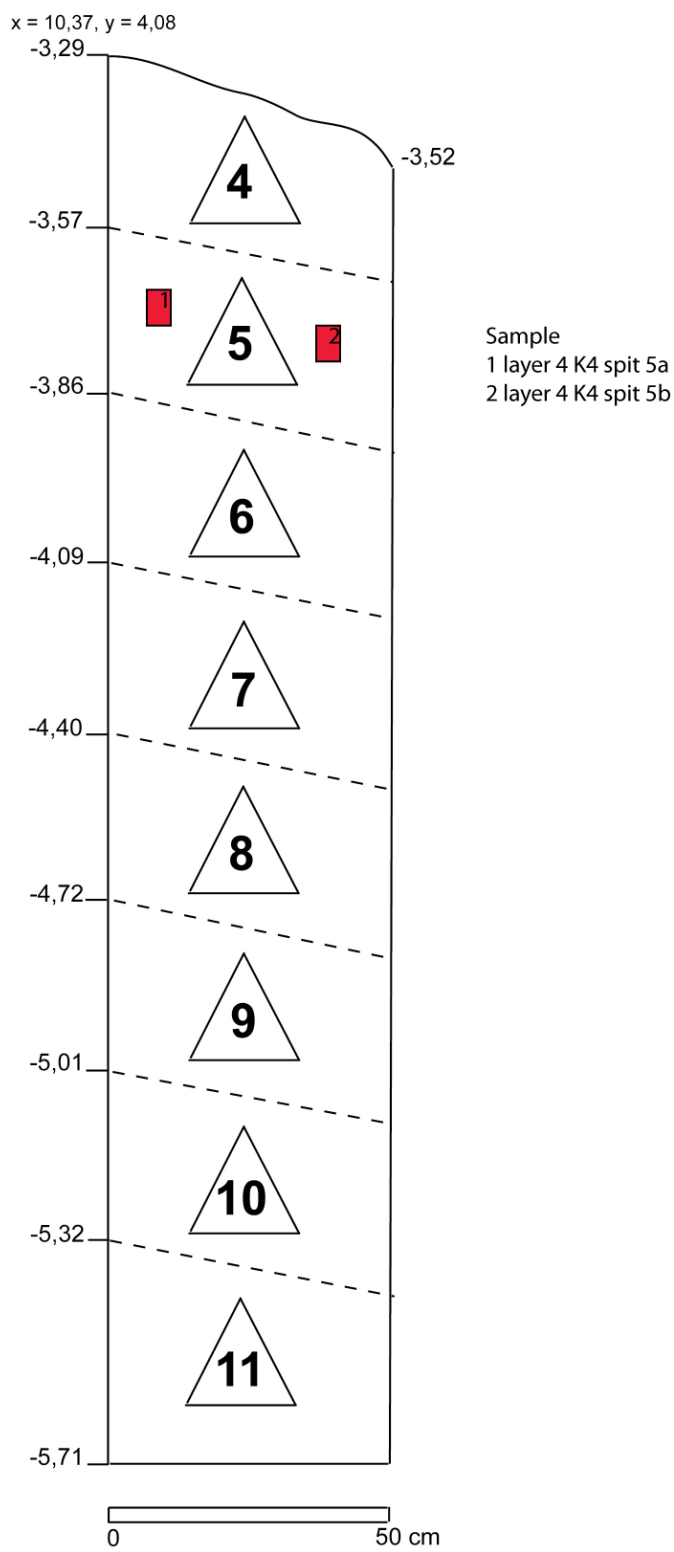
Appendix D: *Profile of samples from Layer 12, redrawn from Pirson (1999)*
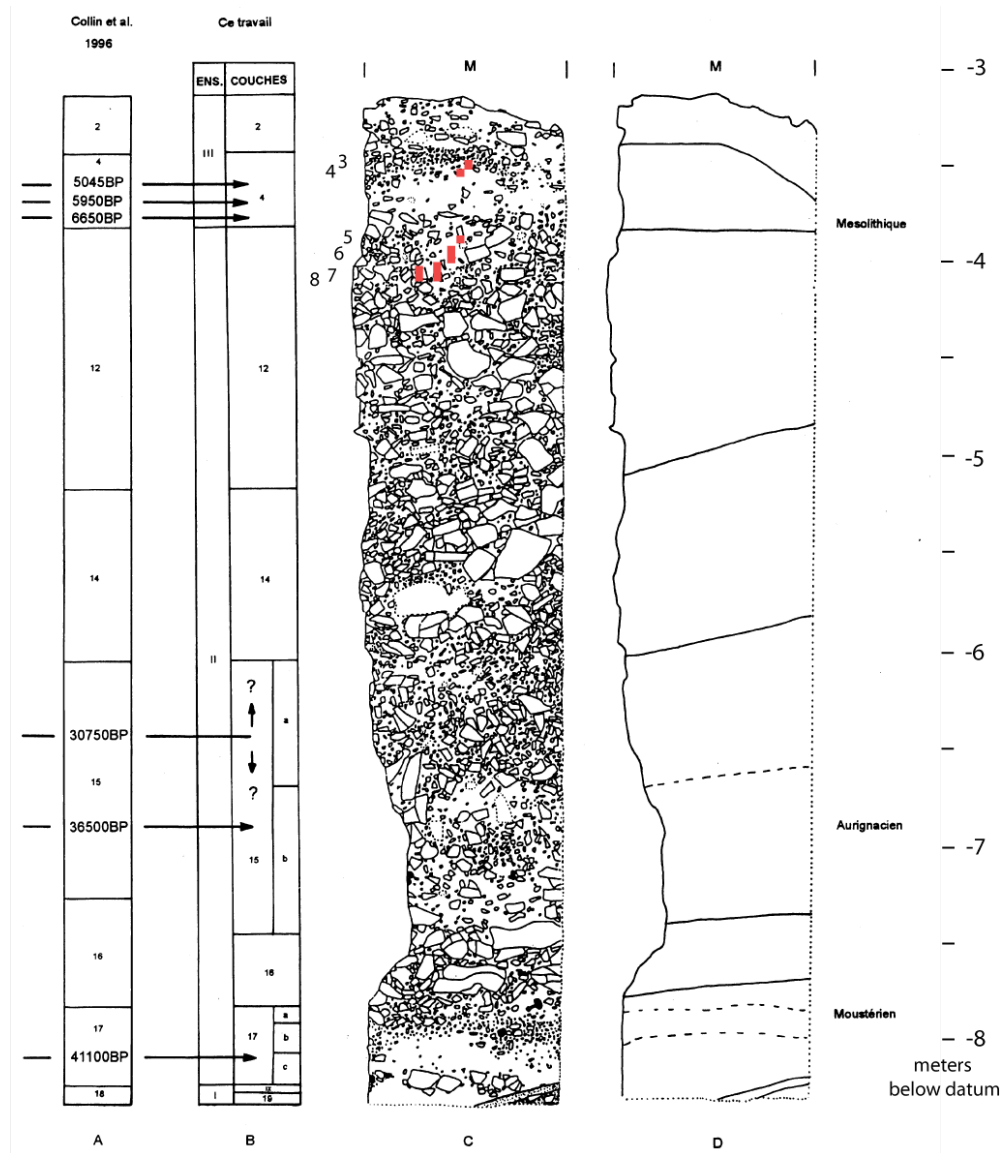
Samples
3 layer 12 M3 spit 1
4 layer 12 M3 spit 2
5 layer 12 M3 spit 9
6 layer 12 M4 spit 5
7 layer 12 M4 spit 6
8 layer 12 M4D spit 6

Samples
9 Layer 14 K6 spit 9
10 Layer 14 K7 spit 9

Note: Due to an error in 2003, squares are numbered differently. K6, using the 2003 numbering, would correspond to K7 using the 1990s numbering. The profile has been numbered according to the original system and the samples placed correctly.

Important:
Sample 9 comes from the base of stratum 12LJ (12, limon jaune), which may be a lateral facies of stratum 14.
Sample 10 comes from near the top of stratum 15.
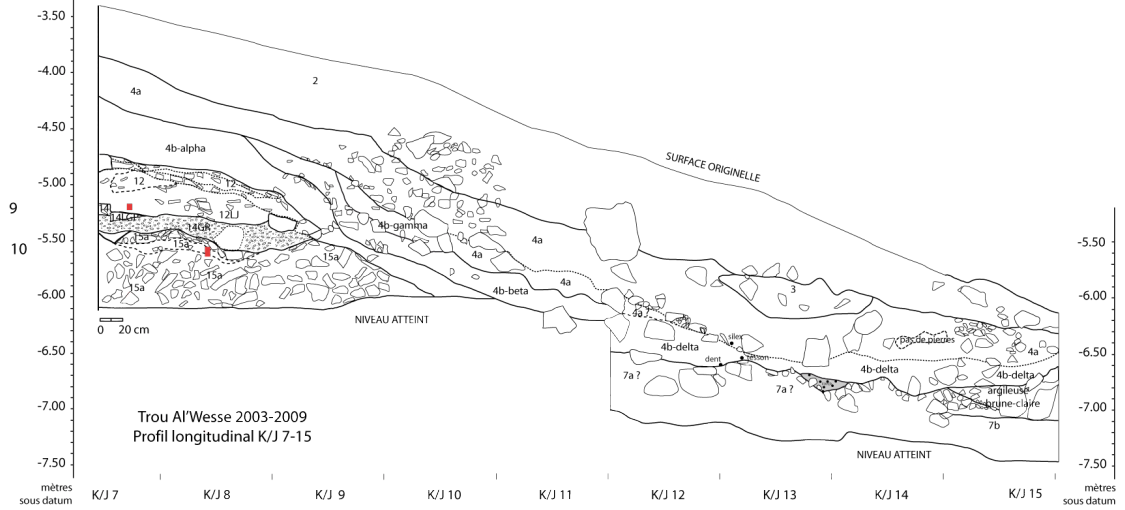
Trou Al'Wesse 2003-2009
Profil longitudinal K/J 7-15

Samples
11 Layer 14 O7 spit 16
12 Layer 16 M7 spit 19
13 Layer 16 M7 spit 16

Given the distance between O7 and the profile, the correct stratum is 14 and not 14-GR as indicated on the profile.

Note: Due to an error in 2003, squares are numbered differently. The L and M squares retained the original numbering, so M7 is still M7, but O7 would correspond to O8 using the 1990s numbering. The profile is numbered according to the original system and the samples are placed correctly.
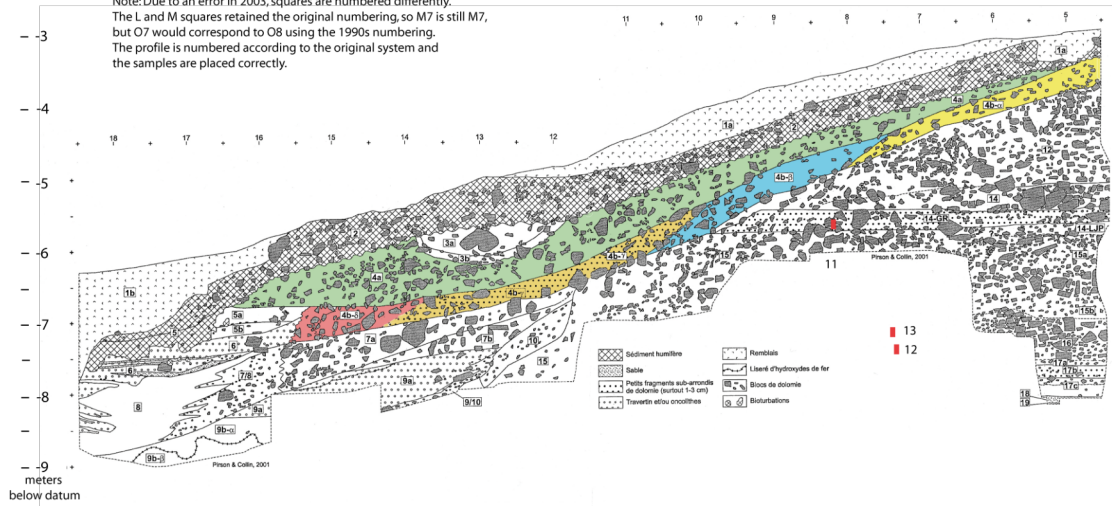
Appendix G: *Arctic collared lemming primer pairs*

Primer Name	Forward Sequence 5' to 3'	Reverse Sequence 5' to 3'	Product Length (bp)	Annealing Temp (0C)
A	CATCTGATACAGCAACAGCATTCTC	GTAGATGCCTCGTCCTACGTGTAA	143	51
B	TAATAGCAACAGCATTCATAGG	CCCCCTCAGATTCATTCTAC	133	50
C	GGGGGCTTCTCAGTTGACAA	GGATTTTGTCTGCGTCGGAG	160	51
D	CTCGGAGACCCAGATAATT	GGAAGGTTATGAGGGCTAG	172	50
E	GCTCCCTACTTGGCCTATG	AGAATATGGAGGCTCCGTTT	171	50
F	GGAGCCTCCATATTCTTCATC	AAGGATATTTGTCCTCATGGG	164	50
G	CTATCAGCAATCCCCTACATC	GTTAGAGCCTGTTTCGTGAAG	171	51
H	GGCTCTAACAACCCATCAGGC	TAAGTGGGTTTGCAGGGGTGT	178	51
I	TAAACTCCGACGCAGAC	TGTGGTGGAGTATTAAGTGG	169	50

Appendix H: *Genbank Sequence Data*

Accession	GI	Genus	Species	Location	Source
AJ131442.1	4499924	Dicrostonyx	torquatus	Russia: East of the Kolyma Delta	Fedorov & Goropashnaya (1999)
AJ131441.1	4499921	Dicrostonyx	torquatus	Russia: West of the Kolyma Delta	Fedorov & Goropashnaya (1999)
AJ131440.1	4499918	Dicrostonyx	torquatus	Russia: Taymyr Peninsula	Fedorov & Goropashnaya (1999)
AJ131439.1	4499915	Dicrostonyx	torquatus	Russia: Yamal Peninsula	Fedorov & Goropashnaya (1999)
AJ238425.1	5763671	Dicrostonyx	torquatus	Russia: East of the Kolyma Delta	Fedorov & Goropashnaya (1999)
AJ238424.1	5763669	Dicrostonyx	torquatus	Russia: East of the Kolyma Delta	Fedorov & Goropashnaya (1999)
AJ238423.1	5763667	Dicrostonyx	torquatus	Russia: Olenekskiy Bay	Fedorov & Goropashnaya (1999)
AJ238422.1	5763665	Dicrostonyx	torquatus	Russia: Taymyr Peninsula	Fedorov & Goropashnaya (1999)
AJ238421.1	5763663	Dicrostonyx	torquatus	Russia: Pechora Bay	Fedorov & Goropashnaya (1999)
AF119275.1	5478475	Dicrostonyx	torquatus	Russia: Kanin Peninsula	Conroy & Cook (1999)
AJ131444.1	4499896	Dicrostonyx	groenlandicus	North West Alaska	Fedorov & Goropashnaya (1999)

Chapter 3

Appendix I: Water vole primer pairs

Primer Name	Forward Sequence 5' to 3'	Reverse Sequence 5' to 3'	Product Length (bp)	Annealing Temp (0C)
A	ACATCTGGTTCTTACCTCAGGG	CCTTGACGGCTATGCTGAGT	173	52
B	TCTCGGGCAGTTGGTATTTTTTA	TATATGTCCCGGATCCATTGATTA	161	52
C	TCACCTTGTCCTGTAGCTGGACTTC	GGGGGTTTGGCAAGCAAAG	209	52
D	CCCAGCTCTGNTAGGCTAAA	TTTCAAGTACACAGGGAGGC	158	51
E	CCCCAAAAACAAGCGAAAC	CAGTGCTTTGCTTTAATATAAGCTACAT	203	50
F	TTACTACCAAAAAACAACTCAAAATTCCA	TATAAGGCCAGGACCAAACCTTTG	158	50

Chapter 4

Appendix J: Hutia primer pairs

Primer Name	Forward Sequence 5' to 3'	Reverse Sequence 5' to 3'	Product Length (bp)	Annealing Temp (0C)
A	CGAAGCTTGATATGAAAACCATCGTTG	GGCATAGGCGAATAGAAAATATCATTCTG	870	54
A2	CGAAGCTTGATATGAAAACCATCGTTG	GCTGTAGATGTGTCTGCGGTGTAATGTA	236	53
B	TTTCGGTTCCTTATTAGGTGTA	CCCTATAAAAGCAGTGGCTATA	295	50
C	TCTTACACTTTTACAGAGACCTGA	TGAATTATTACTATTGCGGTGA	272	50
D	GATTCTTTGCCTTCCACTTTA	CATTCTGGTTTGATGTGAGG	286	51
E	CAGACAACTACACCCCTGC	GCCTCCAATTCATGTAAGGA	263	51
F	CCCTATAAGCCAATGCT	CCAATGTAATTTTTATACTACTAAGAC	214	48

Chapter 5

Appendix K: *Rice rat primer pairs*

Primer Name	Forward Sequence 5' to 3'	Reverse Sequence 5' to 3'	Product Length (bp)	Annealing Temp (0C)
BM2	CCGACACAGCTACAGCATT	TGAAGGATCCGTAGTAAATACCTC	153	52
BM1	TAACTACGGCTGACTAATCCGATA	GTTGTGAGTAATAGGATGATTCCAAT	150	51
G	CATGCTAATGGAGCTTCCATATT	GCCTACGAATGCTGTTGCTAT	144	48
B	ATTTATACTCAACGAAACCTGAAA	CGATGTATGGGATTGCTGA	152	50
BP1	GTAGTTCCGATGTAAGGGA	GGATGAAGTGGAAGGCGAAAAATC	155	54
E	TTTGAGGGGGCTTCTCAGT	AGTAAGGGTGGAATGGGATTTT	180	52
Luc B	TCCTTCATGCTCACTGAAA	GGCTGATAGGAGGTTTGTAAT	141	50
Nev 1	GCCAATGGAGCCTCAATA	GCCTACAAATGCTGTTGCT	141	49
Nev 2	CCTGAAACATTGGAATCAT	GTAGTTCCGATGTAAGGGA	142	49

References

Conroy CJ, Cook JA (1999) MtDNA evidence for repeated pulses of speciation within Arvicoline and murid rodents. *Journal of Mammalian Evolution* **6**, 221-245

Fedorov VB, Goropashnaya AV (1999) The importance of ice ages in diversification of Arctic collared lemmings (*Dicrostonyx*): evidence from the mitochondrial cytochrome b region. *Hereditas* **130**, 301-307.

Grayson DK (2007) Deciphering North American Pleistocene Extinctions. *Journal of Anthropological Research* **63**, 185-214.

Lõugas L, Ukkonen P, Jungner H (2002) Dating the extinction of European mammoths: new evidence from Estonia. *Quaternary Science Reviews* **21**, 1347-1354.

Miller R, Zwyns N, Stewart J, Otte M, Noiret P (2005) La séquence holocène du Trou Al'Wesse : géologie, archéologie et environnement *Notae Praehistoricae* **25**, 129 - 144.

Miller R, Stewart J, Zwyns N, Otte M (2009) The stratified early to late mesolithic sequence at Trou Al'Wesse (Modave, Belgium). In: *Chronology and Evolution within the Mesolithic of North-West Europe: Proceedings of an International Meeting, Brussels, May 30th-June 1st 2007* (eds. Crombé P, Van Strydonck M, Sergant J, Boudin M, Bats M), pp. 287-306. Cambridge Scholars Publishing, Newcastle upon Tyne.

Pirson S (1999) Étude sédimentologique préliminaire au Trou Al'Wesse (Modave, Belgique). *Bulletin de la Société royale belge d'études géologiques et archéologiques (Les Chercheurs de la Wallonie)* **XXXIX**, 115 - 162.

Pirson S, Collin F (2005) Contribution à la stratigra-phie du Trou Al'Wesse à Petit-Modave (comm. de Modave, prov. de Liège. *Notae Praehistoricae* **25**, 39 - 47.

Stuart AJ (2005) The extinction of woolly mammoth (Mammuthus primigenius) and straight-tusked elephant (Palaeoloxodon antiquus) in Europe. *Quaternary International* **126-128**, 171-177.

Stuart AJ, Kosintsev PA, Higham TFG, Lister AM (2004) Pleistocene to Holocene extinction dynamics in giant deer and woolly mammoth. *Nature* **431**, 684-689.

Vartanyan SL, Arslanov KA, Karhu JA, Possnert G, Sulerzhitsky LD (2008) Collection of radiocarbon dates on the mammoths (*Mammuthus primigenius*) and other genera of Wrangel Island, northeast Siberia, Russia. *Quaternary Research* **70**, 51-59.

Veltre DW, Yesner DR, Crossen KJ, Graham RW, Coltrain JB (2008) Patterns of faunal extinction and paleoclimatic change from mid-Holocene mammoth and polar bear remains, Pribilof Islands, Alaska. *Quaternary Research* **70**, 40-50.