

Functional Morphology and Masticatory Biomechanics of
British Red Squirrels

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Thesis Abstract

British red squirrels *Sciurus vulgaris* exist in fragmented populations, with the spread of invasive grey squirrels *Sciurus carolinensis* the leading cause. Populations are isolated, occupying different habitats, consuming different diets and little genetic exchange occurring between them. As such, the potential for rapid evolution in the masticatory apparatus is high. Here, I examine populations of British red squirrels for morpho-functional differences in the masticatory apparatus, while also assessing the mechanical challenges of their diets.

In Chapter 2, I use geometric morphometrics to analyse the cranial and mandibular morphology of four populations of British red squirrels. Variation between populations is revealed, with specimens collected from Formby in the 1990s exhibiting morphological traits suggestive of lesser mechanical abilities of the masticatory apparatus.

In Chapter 3, I employ a novel method to gather data on the material properties of commonly consumed food items. Values of toughness, force to fracture and energy to fracture allows comparisons between food items. It is revealed that the diet of the Formby 1990s population, mostly peanuts, a supplementary food item provided by conservationists, poses a lesser mechanical challenge compared to other food items. A diet of deciduous nuts, like that of squirrels in Northern England, proved the most mechanically challenging.

Chapter 4 attempts to assess the functional abilities conferred by morphological differences revealed in Chapter 2, by assessing biomechanical performance under loading through finite element analysis. This reveals a complicated picture where some models show some ability to perform well under stress and strain, as expected, but despite morphology adapted towards a softer diet, that Formby squirrels still maintain functional abilities.

This thesis assesses the effects on the masticatory apparatus of British red squirrels using a three-pronged approach, revealing how isolation, fragmentation and provision of supplementary foods can influence the morphology, and potential survival, of this native species.

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Author's Declaration

I confirm that this work is original and that if any passage(s) or diagram(s) have been copied from academic papers, books, the internet or any other sources these are clearly identified by the use of quotation marks and the reference(s) is fully cited. I certify that, other than where indicated, this is my own work and does not breach the regulations of HYMS, the University of Hull or the University of York regarding plagiarism or academic conduct in examinations. I have read the HYMS Code of Practice on Academic Misconduct, and state that this piece of work is my own and does not contain any unacknowledged work from any other sources.

A handwritten signature in cursive script that reads "Kim Chalder". The signature is written in a dark ink and is positioned to the right of the main text block.

1. Introduction

Red squirrels *Sciurus vulgaris* are one of the best known and most charismatic animals of British wildlife. Having populated the British Isles since the initial retreat of ice at the end of the last ice age (Shorten, 1954), their presence across the British mainland has ensured a long history of being engrained in British culture and identity. It is therefore surprising that this long-standing British icon has been allowed to reduce in numbers to the point of relative obscurity. The retreat of this native rodent has been much lamented and theories for their disappearance have been heavily explored by researchers (Kenward et al., 1998; Kenward and Hodder, 1998; Kenward and Holm, 1993; Usher et al., 1992; Wauters et al., 2001, Gurnell et al., 2004). Through dedicated research, the introduction of grey squirrels *Sciurus carolinensis* from North America was discovered to be the cause of rapid population decline. It was only early this century (Rushton et al., 2000; Sainsbury et al., 2000; Sainsbury & Ward, 1996; Tompkins et al., 2002) that it was realised that the invader squirrel population did not come alone, bringing the eponymous Squirrel Pox virus, of which against red squirrels had little immune protection. The ensuing rapid population crashes, as disease spreads wherever grey squirrels venture, ensures that in Britain, red squirrels can only survive where grey squirrels are not established (Rushton et al., 2006).

Although of considerable concern for our native squirrels, the fragmentation of this population into small, genetically separated sub-populations offers evolutionary biologists the opportunity to delve into the characteristics of these populations. We can witness first-hand, how fragmentation and isolation in response to an invasive species affects these animals at a micro-evolutionary level. We know from extensive previous work that isolation can accelerate evolution, as the response to selection pressures is magnified (e.g Evans et al., 2012; Lister, 1989; Millien, 2006, 2011). Indeed, these sub-populations of red squirrels can be considered evolutionary islands, encountering similar processes experienced by true island populations. As with Darwin's famous finches, in vertebrates, it is often the masticatory apparatus that is first responsive to selection pressures, with morphological change arising in populations as they adapt to the different mechanical challenges of consuming contrasting diets. With British populations of red squirrels occupying a range of habitats across the UK, the diet of each sub-population is likely disparate, offering opportunities for diverging adaptations. Indeed, first indications of a morphological divergence in the mandible of red squirrels has already been observed (Cox et al. 2020).

The aim of this thesis, therefore, is to further analyse morpho-functional divergence in the masticatory apparatus of British red squirrels, as first explored by Cox et al. 2020. To achieve this aim, I will use the established methods of geometric morphometrics and finite element analysis to investigate morphological divergence between populations and the functional consequences of this divergence. I will also use a self-devised experimental technique to establish the mechanical properties of red squirrel dietary items found across the British sub-populations.

1.2. Taxonomy and Subspecies

Red squirrels are rodents of the family Sciuridae, with sister groups of Aplodontiidae (mountain beavers) and Gliridae (dormice) (Blanga-Kanfi et al., 2009). This suborder has been referred to as Sciuromorpha in past literature (D'Elía et al., 2019), however, this is a confusing term. Sciuromorph is primarily a morphological descriptor of one of the four configurations of the masticatory apparatus of rodents. The sciuromorph condition is not unique to squirrels, having evolved multiple times in the rodent lineage (Emry & Thorington, 1982) and even appearing in non-rodent fossil groups (Ercoli et al., 2019). Rodents such as beavers and pocket gophers are sciuromorphs, but the closest relatives to Sciuridae: Aplodontiidae and Gliridae, are not. As such, it is preferable to use the term Eusciurida for the suborder of Sciuridae, Aplodontiida and Gliridae (Flynn et al., 2020).

Red squirrels do not exhibit sexually dimorphic traits (Shorten, 1954).

1.2.1 Subspecies

Populations of red squirrels have been variously split into many subspecies but the taxonomic validity of most of them is uncertain, with several authors recognizing different numbers. Historically, there have been over 40 subspecies described (Lowe & Gardiner, 1983; Lurz et al., 2005; Sidorowicz, 1971). These were initially designated based on morphology, most often coat colour, and geographical isolation (such as in (Kerr, 1792)).

1.2.2 The British Subspecies

A subspecies of red squirrel, *Sciurus vulgaris leucorouus*, was once thought to exist in Britain and Ireland (Sidorowicz, 1971). This subspecies was named by Kerr (1792), based on distinct morphological traits described by Pennant (1781). Characteristics included 'bleaching', where the fur of the ear tufts and tip of tail become white in the summer, and skulls that are smaller than their European counterparts. Bleaching was considered to be particularly

diagnostic, as mainland European red squirrel populations do not experience this, instead having distinctive coat-colour polymorphism (Wauters et al., 2004) with dorsal melanistic, red and brown coat-colour polymorphic variants, which may be an adaptive trait (Hale & Lurz, 2003). Hale et al. (2001) investigated coat colour polymorphism in squirrel populations in Northern England. It was noted to be present in northern English squirrel populations, and the prevalence of the bleaching trait has diminished. However, the bleaching trait was identified in individuals from Cumbria before 1980 – suggesting there may have been significant genetic mixing of populations since the establishment of Kielder Forest (Barratt et al., 1999). Lowe & Gardiner (1983) proposed that the bleaching trait only occurred in squirrels in East Scotland, Ireland and Thetford. In Ireland, the bleaching trait is common but not ubiquitous, recorded in 57% of individuals studied (Finnegan et al., 2009).

Studies into skull shape could not show any difference between populations in the UK and Europe in specimens collected post 1935, with only 38% of specimens pre-1935 considered ‘distinct’ in shape (Lowe & Gardiner, 1983). Grill et al. (2009) performed geometric morphometrics on mandible shape of all the purported subspecies, but no diagnosable difference was found, except for in *S.v.infuscatus* (native to the Iberian peninsula) and an extinct 19th century population from Dorset, England.

Genetic analysis to date does not support the existence of an extant British subspecies. Presence of only three subspecies was found based on mitochondrial phylogroups – *S.v.fuscoaster* in Eastern Europe, and *S.v.infuscatus* and *S.v. meridionalis* in Italy (Wauters et al., 2017). Subsequent genetic and morphological analysis of *S.v. meridionalis*, has led to the description of a separate species entirely, *Sciurus meridionalis* (Barratt et al., 1999). In the UK, studies on mitochondrial DNA (Hale et al., 2004), did not detect any genetic distinction between British and mainland Europe populations. A further mtDNA study (Hale et al., 2004) indicated that most extant populations of red squirrels in Britain today have mainland European ancestry, identifying a Scandinavian haplotype that started to appear in specimens between 1966 – 2002 (Hardouin et al., 2019). Additionally, no clear genetic difference between the populations in Ireland and mainland UK could be detected (Marr & Macleod, 2019).

The populations of red squirrels in Britain and Ireland have a complicated history with several historical local extinctions (such as in Ireland and Scotland, see Section 3) and subsequent introductions from squirrel populations in mainland Europe. As such, it seems

likely that if there ever were a British/Irish subspecies, the genetic mixing with other subspecies from mainland Europe, combined with extinctions, has led to British and Irish populations having little genetic or morphological distinctiveness. Genetic studies have shown that gene flow between mainland UK and mainland Europe populations may have been occurring up until 8000 years ago (Lurz et al., 2005). As such, if there was a genetically or morphologically distinct *S.v. leucorouus* subspecies, it evolved after the disappearances of land bridges between the UK and mainland Europe and has since become extinct.

1.3. History of Red Squirrels in Britain

Great Britain is one of the few places of its native range where red squirrels could be considered endangered (Gazzard, 2023). Although widespread and abundant across Europe, their populations have dwindled in Britain since the late 1800's. The cause of this decline was much debated, with habitat destruction, urbanisation and competition with grey squirrels considered possible reasons (Gurnell et al., 2004b; Kenward et al., 1998; Kenward & Hodder, 1998; Kenward & Holm, 1993; Usher et al., 1992; Wauters et al., 2001). It was only when Squirrelpox virus (SQPV) was discovered and named, in the early 2000s, that this disease, spread by greys and largely fatal to red squirrels, was acknowledged as the primary cause of population decline century (Rushton et al., 2000, 2006; Sainsbury et al., 2000; Sainsbury & Ward, 1996; Tompkins et al., 2002). The nature of SQPV means that outbreaks are aggressive, causing mass fatalities among red squirrels soon after being carried into an area. This means today, red squirrel populations survive only where grey squirrels have not been able to spread (islands such as Jersey, Isle of Wight and Brownsea Island) or where extensive grey squirrel management projects are in place (such as populations in Northern England, Scotland and Wales) (Rushton et al., 2006). Indeed, some populations existed until relatively recently through conservation projects, until finally dwindling to unsupportable numbers (such as a population in Thetford forest, see Section 4). The ongoing conservation of red squirrels in Britain is facilitated mainly through efforts to prevent the spread of the SQPV, with some work done in supplementary feeding. Conservation groups include both governments funded and charity supported organisations, with many groups being entirely volunteer based. Details of the different groups working to protect red squirrels in Britain can be accessed in books published by the Red Squirrel Survival Trust and the European Squirrel Initiative: Saving the Red Squirrel - Landscape Scale Recovery (Shuttleworth et al., 2021) and Shared Experience of Red Squirrel Conservation Practice (Shuttleworth et al., 2015). The history, extent and structure of British populations of red squirrels is explored below.

1.3.1 North Scotland

Most red squirrels in Britain today are found in Scotland and Northern England. Within Scotland populations are found mainly in the Highlands where grey squirrels have not yet arrived, but also some populations in the North East and South below the central belt (Gurnell et al., 2014a; Shuttleworth et al., 2021). Red squirrels are thought to be indigenous to Scotland but suffered an apparent decline in the 17th and 18th centuries, prior to grey squirrel introductions, and were later reintroduced to Scotland ten or more times between 1772 – 1872 from England (Shorten, 1954). The origin of these translocated squirrels is unclear but at least one introduction was from Norway or Sweden to Perthshire (Barratt et al., 1999; Harvie-Brown, 1880; Lurz, 2010). Red squirrels then became so abundant that they were considered pests, causing damage to conifer plantations and were subject to reduction campaigns by organisations such as the Highland Squirrel Club (1903 – 1946) (Lloyd, 1983).

Today, red squirrel protection in Scotland is based on grey squirrel control in accordance with the Scottish Strategy for Red Squirrel Conservation (2015), and facilitated by *Saving Scotland's Red Squirrels* (hereafter SRSS)(Shuttleworth et al., 2021). Particular focus of grey squirrel control has been aimed at the 'Highland Line' a 10km wide band between the east coast of Scotland (north of Montrose), through Kirriemuir, Crieff and Loch Lomond to the west coast at the Firth of Clyde near Helensburgh (Collis & MacMaster, 2021). The goal of this project is to prevent grey squirrels spreading north into the Highlands which serves as a red squirrel 'stronghold' and has never had grey squirrels before (Shorten, 1954). SRSS also have projects focusing on Aberdeenshire, where an 'island' population of grey squirrels exists (Woodfin, 2021). Grey squirrels were introduced to Aberdeen in the 1970s and are now abundant in the city. Their spread into surrounding areas has been a cause for concern, particularly to the relatively nearby red squirrel populations in the Cairngorm National Park. Focused eradication work by SRSS has been helpful in the overall recovery of red squirrel numbers in rural Aberdeen. SRSS continues action in urban areas with the aim of total eradication of greys. In North-West parts of the Highlands, near Inverness, red squirrels were once abundant with large scale deforestation after WW1 and WW2 and persecution from the Highland Squirrel Club blamed for the decline (Prestley, 2021). This area has been identified as a region for reintroduction with the lack of grey squirrels and wide-spread afforestation creating suitable habitat. Nine translocations have been carried out so far involving 180 squirrels trapped from areas within a 40-mile radius of Inverness (Priestley, 2021). All reintroduced populations are still present as of 2020.

1.3.2 South Scotland and Northern England (Border Region)

The central belt of Scotland is bereft of red squirrels, likely due to the large urban areas of Edinburgh and Glasgow covering this region. Here, grey squirrels are likely better competitors than reds, which prefer the pine forests of the highlands (Gurnell et al., 2004b). However, south of this central belt, there are surviving red squirrel populations, despite the presence of grey squirrels. The Scottish Wildlife Trust considers them to be thriving in Tweeddale and comfortably present in the catchments of Teviot, Borthwick and Ettrick waters. Although under pressure from grey squirrels, red squirrels are also considered to be thriving in Dumfries and Galloway (Sinclair et al., 2021). Initial introductions of grey squirrels to South Scotland in the early 20th century did not bring SQPV, allowing relatively competition-free coexistence. However, in 2008, grey's seropositive for SQPV were detected at locations close to the border with England as carrier greys spread north. Greys have now largely replaced reds in some areas, such as Berwickshire (Garson, 2021).

Red squirrels also exist in parts of Northern England, being most abundant in Cumbria and Northumberland (Parrott et al., 2009). In 2005, Red Squirrels Northern England (RSNE) designated 17 large conifer forests as red squirrel reserves, 10 in Northumberland, 5 in Cumbria, 2 in North Yorkshire and 1 in Merseyside (Traut, 2021). Most of these populations have both red and grey squirrels and are subject to constant conservation work, although with varying levels of government support (RSNE, 2023). Although there are no known introductions of red squirrels from outside Britain into Northern England, most squirrel populations here show recent (within the last 40 years) Scandinavian ancestry (Hale et al., 2004). This is despite 'Scandinavian haplotypes' not existing in British squirrels until 1966. Red squirrels in Cumbria were thought to be remnants of original British red squirrel populations, with no historical evidence of European introductions and coat colours characteristics described as common in 18th century English populations (Shorten, 1954). However, the 'Scandinavian haplotype' is now dominant in all northern English populations. Hale et al. (2004), suggest that the Scandinavian haplotypes expanded into Cumbria only very recently, and the reason for its widespread expansion is potential adaptive advantage in the non-native spruce plantations of Northern England. Indeed, the creation of non-native timber plantations has allowed for dispersal of Northern English populations (East Cumbria and North Northumberland) within the north of England and South Scotland (Hale et al., 2001, 2004). The Kielder Forest is a plantation of Sitka spruce *Picea sitchensis*, Norway spruce *Picea abies* and lodgepole pine *Pinus Contorta*. At 50,000ha, it straddles the Scottish-

English border with 78% of the forest in Northumberland National Park and 22% in Cumbria. The maturing trees likely allowed for dispersal of red squirrel populations northwards to south Scotland with genetic analyses showing that the establishment of this forest has facilitated the dispersal of Cumbrian genes north, spreading among the Scottish populations (Hale et al., 2001).

As the populations of Southern Scotland and Northern England are connected through the Kielder Forest, I can consider squirrels living in this area as a single population, with dispersal across Northern England and South Scotland likely. Therefore, for simplicity, I will refer to this population as the 'Border Region' or the 'Borders' population throughout the rest of this thesis.

1.3.3 Northern England - Formby and Sefton Coast

On the Sefton Coast, in North Merseyside/West Lancashire, red squirrels are widespread but most concentrated in the coastal reserve woodlands of Formby and Ainsdale (Cripps, 2021). The Formby woodland is of particular focus of conservation work by the Lancashire Wildlife Trust. This woodland, owned by the National Trust, provides habitat to a high-density population of red squirrels. (Gurnell & Pepper, 1993; Rice-Oxley, 1993). The site is approximately 70 hectares and consists of a mix of habitats including mixed coastal, sand dune forests and pine forests with red squirrels also existing in suburban areas surrounding the woodland (Chantry et al., 2014; Gurnell & Pepper, 1993; Miller, 2015). The 40 hectares of pine forests is dominated by Corsican and Scots Pine but also has a mix of deciduous scrub (primarily sycamore) and small patches of lodgepole pine (Shuttleworth, 2000). This population likely stems from an introduction of red squirrels from Europe to Ainsdale around 1933 (Gurnell & Pepper, 1993).

Red squirrels here are of particular interest as populations have been encouraged to persist through supplementary feeding. The site has had a grey squirrel control scheme in place in urban areas since 1993, which was expanded in 2005 (Chantry et al., 2014). However, this has not prevented an outbreak of SQPV in 2007 and 2018 (Cripps, 2021). Populations were decimated by the virus in 2007 and declined by 87% between Autumn 2007 and March 2009 (Chantry et al., 2014; Miller, 2015). Fortunately, by 2010 numbers had recovered to more than half of their previous population size (Miller, 2015). The impact of the 2018 outbreak is yet to be fully evaluated.

1.3.4 Southern England – Brownsea Island, Isle of Wight and Recently Extinct Populations

There are very few red squirrel populations in southern England and where populations do exist, they are low in numbers (Gazzard, 2023). Three of these populations are Island populations that are protected by natural barriers to the spread of grey squirrels. Two islands are in Poole Harbour: Furzey and Brownsea Island; as well as the Isle of Wight.

Furzey and Brownsea Islands both have small but stable populations of red squirrels, in mixed deciduous forests naturally protected from grey squirrel colonisation. Furzey Island has a very small population of around 30 individuals (Thain & Hodder, 2015). The island is only 13ha with six hectares of woodland dominated by Scots pine. The Furzey island population are most likely to have a Northern English origin, being introduced to the island in 1977 from Cannock Chase, Staffordshire (Kenward & Hodder, 1998). Brownsea is a larger island of 250 hectares only 300 metres from Furzey. The population of red squirrels on this island is known to have been established prior to the Furzey Island population (Thain & Hodder, 2015). However, migration between the islands is feasible and it has been shown that they share haplotypes (Hardouin et al., 2019). Introduction of the Brownsea population is unrecorded and is considered a remnant native population. There is a known red squirrel population of roughly 200 individuals and several student projects have been conducted here in conjunction with Bournemouth University (Thain & Hodder, 2015). Conservation efforts to promote woodland structure establishment has led to a habitat composed of Scots and Maritime pine *Pinus pinaster*.

The Isle of Wight boasts the largest island population of red squirrels and is considered another stronghold for red squirrels in Britain. While genetic diversity among the Brownsea and Furzey Island populations is low, the Isle of Wight showed genetic diversity like that of non-island populations, with no genetic bottleneck detected (Hardouin et al., 2019, 2021). However, there appears to be little genetic mixing between populations on the east and west of the island (Hardouin et al., 2021). Numbers are estimated to be around 3300 (Pope & Grogan, 2003). Their introduction to the island is not documented but genetic analysis has shown they share haplotypes with Southern English populations that no longer exist (Hardouin et al., 2019). Woodland habitats on the island are fragmented due to development (Wight Squirrel Project and The Isle of Wight Red Squirrel Trust., 2015) and are comprised of oak and hazel (Kenward & Hodder, 1998). Squirrels have easy access to supplementary foods, provided by people in gardens (Butler, 2021; Wight Squirrel Project and The Isle of Wight Red Squirrel Trust., 2015). There are no grey squirrels on the Isle of Wight (Wight Squirrel Project and The Isle of Wight Red Squirrel Trust., 2015).

Most populations across the rest of the south of England have gone extinct – for example, a small population existed in Thetford Chase in East Anglia (Gurnell et al., 1997; Gurnell & Pepper, 1993). This site was a red squirrel reserve dominated by Scots and Corsican pine, oak and beech trees (Gurnell et al., 1997). The grey squirrels' arrival in the 1980's is a likely factor in the extinction of the reds, despite a grey squirrel trapping programme between 1992-1996 (Gurnell et al., 1997, 2002). An additional factor is food availability. The site was commercially planted with pines in 1925-1940. Young Scots pine produces good cone crop, so reds were common in the 1950-60s coinciding with good food supply. However, as the trees aged there was less food available (Gurnell et al., 2002). Population was as low as 10-20 individuals in 2002 (Gurnell et al., 2002) and is now thought to be extinct (Matthews et al., 2018). However, there are a small number of captive-bred red squirrels in East Anglia, managed by members of the East Anglican Red Squirrel Group (Kelley, 2015), which presumably originate from the Thetford population. In captivity they have been fed a diet of nuts, seeds, cucumber, sweetcorn and deer antler (Kelley, 2015).

1.3.5 Wales

Although, in the past, red squirrels were prevalent across much of Wales (Lloyd, 1983; Shorten, 1952), numbers are now low but do exist in a few areas. In 1995 they were estimated at around 10,000 animals (Harris, 1995) but today are probably lower than 1000 animals. Areas of Wales which have small populations of red squirrels include Anglesey and its closest point on the mainland, Gwynedd (Hobbs, 2005; Shuttleworth, 2015; Shuttleworth et al., 2015) as well as Clocaenog forest, Denbighshire and small pockets in central Wales (Hobbs, 2005; Mason & Young, 2021; Wilberforce et al., 2015).

The Isle of Anglesey currently hosts the largest population of red squirrels in Wales, with estimates in 2015 of around 700 individuals (Shuttleworth, 2015). Anglesey could be a haven for red squirrels due to the natural sea barrier to grey squirrel invasion, only connected to the mainland via two bridges. However, grey squirrels did arrive here in the mid 1960's which led to a push back of red squirrels into the spruce and pine dominated Mynydd Llwydiarth forest plantations, near Red Wharf Bay (Shuttleworth, 2015). Several red squirrel releases occurred from 2004-2008, along with an intensive grey squirrel capture programme, which has led to a rise in numbers. Grey squirrels were considered fully eradicated from the Island in 2013 (Shuttleworth, 2015). Red squirrels are now known to have crossed back onto the mainland and are found in some wooded areas in Gwynedd (Shuttleworth et al., 2015). Grey squirrel

eradication programmes have also taken place on the mainland to protect Anglesey populations from grey squirrels re-crossing to the Island (Shuttleworth et al., 2015). Habitat on the island utilised by red squirrels is a mix of broadleaf and coniferous woods but supplementary feeding also takes place in gardens, encouraged by the provision of wooden feeding boxes by the Island Haven Project (Shuttleworth et al., 2015).

Clocaenog forest is a large (5500 hectare) commercial conifer plantation in northeast Wales. It was planted in the 1930s and provided habitat for a population of up to 400 red squirrels until recently, where numbers have dwindled to around 50 animals (Mason & Young, 2021). Although in very low numbers, and difficulty in estimating populations (Lloyd, 1983), there is also a small population of red squirrels in Mid Wales (Hobbs, 2005; Wilberforce et al., 2015). Numbers are thought to be between 100 and 500 individuals (Wilberforce et al., 2015) and are mainly found in coniferous plantations in the upper Twyi Valley, Cambrian Mountains (Hobbs, 2005; Wilberforce et al., 2015).

Results of genetic studies on Welsh populations of red squirrels suggested that squirrels across Wales were more closely related to each other than other British populations, with potential lineage to an ancestral Welsh population (Hobbs, 2005; Ogden et al., 2005).

1.3.6 Jersey

In the Channel Islands there is a population located on the Island of Jersey. Jersey, like the Isle of Wight, does not have any grey squirrels and has escaped mortalities caused by squirrelpox (Blackett et al., 2018; Simpson et al., 2010). Jersey red squirrels were thought to be introduced to the Island both from southern English populations that no longer exist, and from mainland Europe (Le Sueur, 1976). This is supported by genetic analysis (Simpson et al., 2013). Habitat utilized by red squirrels is comprised of sycamore *Acer pseudoplatanus*, pedunculate oak *Quercus robur* and sweet chestnut *Castanea sativa* (Magris & Gurnell, 2002). This type of woodland covered only 540 ha of the island (116 km²) in 2002 (Magris & Gurnell, 2002) and is highly fragmented with most of the island classified as urban. However, squirrels are found across the entire island (Simpson et al., 2013) and make use of supplementary food left out by the public (Magris & Gurnell, 2002).

*1.3.7 The Grey Squirrel *Sciurus carolinensis*, an invasive congener*

Along with Italy and Ireland, the UK is one of few places where grey and red squirrels exist sympatrically. Grey squirrels are native to North America (Koprowski, 1994) but were first introduced into Britain in 1890, to Woburn, Bedfordshire and in 1892 to Argyll and were released several more times between then and 1920, by which time they were established in at least 12 different locations across Britain (Middleton, 1930; Shorten, 1952). In Ireland they were first introduced to Longford in 1911 and by 1996 had spread over the eastern half of Ireland (Ó'Teangana et al., 2000) and all counties of Northern Ireland (Ó'Teangana et al., 2000). Since then, red squirrel numbers in the UK and Ireland have suffered a significant decline, with replacement of reds with greys in all areas affected (Rushton et al., 2000).

Many theories were explored in reasons for the decline of red squirrels. Greys are not generally aggressive towards reds in shared habitats, appearing to be relatively mutually tolerant (Wauters & Gurnell, 1999). Reproduction rates of female reds were not reduced significantly in the presence of grey squirrels (Gurnell et al., 2004). Greys appear to have some competitive advantage in food availability and are capable of exploiting food crops while still unsuitable for reds, (e.g. hazelnuts which they can consume early in the season when still indigestible to red squirrels) (Kenward & Holm, 1989). Red squirrels are therefore left with relatively less food for winter survival. Greys have been shown to put on more body mass in the autumn than reds (Kenward & Tonkin, 1986), potentially increasing their ability to survive over the winter period. In areas of deciduous forests where greys and reds coexist, greys appear to have a competitive advantage, evidenced by the replacement of reds with greys across many mixed deciduous forests in the UK (Gurnell et al., 2004; Kenward et al., 1998; Kenward and Hodder, 1998; Kenward and Holm, 1993, 1989; Kenward and Tonkin, 1986). Red squirrels appear to be better adapted towards foraging in conifer forests - they are of slighter build, allowing them to be adept at moving amongst the light branches high up in conifer trees (Kenward & Tonkin, 1986), while greys appear to be better generalists. There is lower red squirrel recruitment in the presence of greys in mixed deciduous forests (Gurnell et al., 2004) and studies have shown that reds are unlikely to persist in grey-populated forests with more than 14% oak canopy (Kenward & Holm, 1989). However, difference in food exploitation ability was not considered a universal explanation (Wauters et al., 2001)

Later studies show that it was disease-mediated competition predominantly responsible for the decline of red squirrels (Atkin et al., 2010; Bruemmer et al., 2010; Chantry et al., 2014; Parrott et al., 2009; Rushton et al., 2000; Tompkins et al., 2002). Squirrelpox is a viral skin disease that is largely asymptomatic in grey squirrels but almost always fatal when contracted

by reds (Tompkins et al., 2002), with only 8% of reds likely to survive once infected (Chantry et al., 2014). The reasons for this are currently unknown (McInnes et al., 2013). It is now well-documented to be involved in numerous rapid population declines and extinction events across the UK (Chantry et al., 2014; Rushton et al., 2000, 2006; Sainsbury & Gurnell, 1995; Sainsbury & Ward, 1996; Tompkins et al., 2003). Areas that had shown no previous history of infections, such as Ireland and Scotland, both showed confirmed cases of SQPV in red squirrels relatively soon after seropositive grey squirrels were detected in the area (McInnes et al., 2013; McInnes et al., 2009), indicating that greys are the main vector of the disease. Further support for this hypothesis is seen in island populations without grey squirrels, such as Jersey, having had no recorded SQPV infections (Blackett et al., 2018; McInnes, 2018; Simpson et al., 2010). Once contracted by red squirrels, infection is transmitted quickly within the population as infected skin tissue is shed, quickly causing an epidemic with mass mortalities. With grey squirrels acting as a reservoir for the virus, mixed squirrel areas are particularly vulnerable to localised extinction of red squirrels (Bruemmer et al., 2010). While SQPV outbreaks have likely been occurring in Wales and mainland England for many decades, the first identified squirrelpox case was in Norfolk in 1981 (Everest, 2021; Scott et al., 1981). Scotland recorded its first confirmed SPQV red squirrel infection in 2007 (McInnes et al., 2009). SQPV spread by grey squirrel invasion was likely the final nail in the coffin for populations in most of England, already suffering due to habitat loss.

The spread of SQPV further into other red squirrel populations, particularly on mainland Europe, is of major concern. A population of grey squirrels exists in Northern Italy, stemming from an introduction to Piedmont in 1948 (Currado et al., 1987). Predictions of rapid expansion (Lurz et al., 2001; Tattoni et al., 2004), have not come to fruition, and although still expanding, grey squirrel spread is much slower than in Britain and Ireland (Bertolino et al., 2014). No SQPV has been detected in Italian grey squirrel populations (Romeo et al., 2019). Without SQPV facilitating replacement, grey squirrels have not experienced the rapid expansion witnessed in Britain and Ireland.

1.4. Ecology – Habitat and Diet

As discussed in Section 4, the spread of invasive grey squirrels throughout Britain has significantly impacted the current distribution of red squirrels. Where grey squirrels are present red squirrels rapidly die out due to spread of the highly fatal SQPV, of which greys are carriers. This had led to a distribution where populations only exist where they are

protected from grey squirrel encroachment either by physical barriers, such as on islands like Jersey, Isle of Wight, Brownsea and Anglesey, or where buffer zones of red squirrel eradication are in place such as Formby, Northern England and Scotland (a full list of British Red Squirrel populations as of 2023 is listed below in Table 1.1) . Fragmentation means these populations are genetically isolated from each other with little opportunity for dispersal. Additionally, these populations occupy habitats ranging from native pine stands, non-native conifer plantations, mixed conifer-deciduous woodlands and suburban areas. Red squirrels consume a diet of pine seed, nuts and occasional secondary foods such as conifer buds, fungi and the cambial tissue of trees. With the availability of nuts and seeds dependent on the types of trees present in a woodland, the diet of British red squirrel populations is likely to be highly variable.

1.4.1 Woodlands across Britain

The woodland habitats across Britain vary depending on climate and history of the area. The forests of the Scottish Highlands comprise mostly of native Scots pine *Pinus sylvestris*, with some timber plantations bringing in non-native conifer species. In Northern England, there are pockets of mixed woodland with hazel *Corylus avellana*, oak *Quercus sp* and yew *Taxus baccata* (Hale & Lurz, 2003; Tonkin, 1983), but also large areas dominant in non-native conifer species. The Kielder forestry plantation, 22% of which is in Cumbria and 78% of which is in Northumberland National Park comprises of Sitka spruce *Picea sitchensis*, Norway spruce *Picea abies* and lodgepole pine *Pinus contorta* (Hale & Lurz, 2003). This plantation links woodlands of eastern Cumbria, south Scotland and north Northumberland. Formby national park is predominately Scots pine but also some lodgepole and Corsican pine *Pinus nigra* (Shuttleworth, 2000). In Southern England, forests are more commonly deciduous-mixed forests such as the oak, beech, Scots and Corsican pine woods of Thetford where red squirrels are now locally extinct (Gurnell et al., 1997). The Isle of Wight is mostly oak-hazel woods, although highly fragmented and the area is mostly sub-urban (Butler, 2021; Kenward & Holm, 1993). Brownsea island is mostly conifer, comprised of Scots pine and maritime pine *Pinus pinaster* (Thain & Hodder, 2015), while nearby Furzey island is mostly mature Scots pine with some peripheral oak and sweet chestnut *Castanea sativa* (Kenward & Hodder, 1998). On Jersey, woodlands comprised of sycamore *Acer pseudoplatanus*, pedunculate oak *Quercus robur* and sweet chestnut *Castanea sativa* cover only 116km² of the island (as of 2002: Magris & Gurnell, 2002) with the rest of the island classified as urban. In Wales, red squirrel inhabited woodlands are mainly mixed-coniferous forests such as

Clocaenog, which is 69% Sitka spruce with some European larch *Larix decidua* and Norway spruce (Mason & Young, 2021). Anglesey has forests of mixed Scots pine and spruce species (Shuttleworth, 2015). See Table 1.1 for full list of red squirrel occupied British woodlands.

1.4.2 Diet

Pine seed extracted from cones appears to be the preferred diet of red squirrels. A review of stomach content analysis and observations of red squirrel feeding across the UK and mainland Europe (Kenward & Holm, 1993) showed that conifer seeds are most prevalent in the diet. 97% of sightings of red squirrels feeding in East Scotland, were observed feeding on pine seeds (Kenward & Holm, 1993) and pine seeds were found in the stomach contents of 100% of 52 squirrels dissected in Scotland (Vickery, 1984). Squirrels may even consume a large portion of the cone crop before the cones are fully opened (Moller, 1986). The part of the cone they are consuming is the pine seed endosperm. To extract this, squirrels have a distinctive method of accessing the seeds, using their forepaws to grip the cone at each end and remove the sterile and rudimentary bracts at the blunt end of the cone. Each scale is removed from the cone's central axis, beginning at the blunt end, and the cone rotated, moving towards the tip where the seed-bearing bracts are concentrated (Lurz & South, 1998). This leaves the central part of the cone with the bracts carefully removed. These dismantled cones are often used by ecologists as an indicator that squirrels are in the area. Although small, pine seeds have a relatively high energy content per weight (Shuttleworth, 1997), easily satisfying the minimum dietary requirement of red squirrels, calculated at 10-12% crude protein and 16-17% crude fat (Pulliainen, 1984).

In addition to pine seeds, deciduous nuts are regularly consumed by red squirrels. Hazelnuts, acorns, beechnuts and sweet chestnuts are varyingly available in deciduous woodlands. Yew seeds are also consumed in mixed deciduous-coniferous woodlands. Red squirrels generally focus on hazelnuts over other available nuts (Kenward & Holm, 1993). Acorns, although often associated with red squirrels in popular culture, are consumed in relatively low levels even when crop abundance is high (Kenward & Holm, 1993). Acorns contain polyphenols (tannins) that are toxic to many mammals, possibly interfering with absorption of nutrients and water in the gut of red squirrels (Kenward & Holm, 1993). In captive squirrel studies, reds chose not to consume acorns if offered a mixed diet, and, when no alternative was offered, their body mass decreased (Wauters et al., 1995). Grey squirrels appear to have a higher tolerance to the phytotoxic effect of acorns, consuming more acorns than reds

(Shuttleworth, 2000). More effective acorn exploitation, in addition to the willingness of grey squirrels to consume hazelnuts in earlier stages of ripeness than red squirrels, suggests that in mixed hazel-oak forests, grey squirrels are likely to be better competitors than red squirrels. Indeed, the different abilities to digest acorns was suggested as a reason for grey squirrels out-competing reds in mixed woodlands before the extent of disease-mediated replacement was understood (Kenward & Holm, 1993).

Rodents have been shown to have preference for foods that have the highest caloric gain per unit feeding time (Robb et al., 2008a), which may affect squirrel's choice of nuts depends how difficult they are to remove from the shell. When these high calorie foods are less available, secondary foods are consumed. For red squirrels, these include conifer buds, flowers and shoots, fungi, catkins of deciduous trees, bird eggs and occasionally bones and antlers are gnawed (Moller, 1983; Shorten, 1954). The cambial tissues of trees (layer under bark) is also eaten, with young trees more commonly targeted (Shorten, 1954), which can cause damage in timber plantations (Krauze-Gryz & Gryz, 2015). Fungi are energetically cheap to find and eat and relatively rich in nitrogen and other minerals so become an important food source when pine crops are less abundant (Grönwall & Pehrson, 1984).

1.4.3 Supplementary feeding

Food supplementation is occasionally used in conservation practices, in attempts to improve breeding success and survival of endangered species (Shuttleworth, 2000). This can be an effective conservation technique that helps buffer the effect of seasonal availability of preferred food and can be used to attract red squirrels to an area (Shuttleworth, 1997). In areas where supplementary food has been provided, such as Formby, squirrels often have high density populations. However, when provided without sufficient research, negative effects can occur such as behavioural changes, nutritional deficiencies or spread of disease at feeding stations (Shuttleworth, 2000). In some cases, the provision of human-grade food to animals has resulted in the accidental damage to immune systems via consuming antibiotics or pesticides in human food (Kostrzewa & Krauze-Gryz, 2020).

In Formby, Merseyside, squirrels were provided supplementary food in the form of peanuts for approximately 10 years. Between 1994 and 1996, an excess of 2500kg of peanuts were supplied annually (Shuttleworth, 1997). Despite this, pine seed still made up most of the diet (65%) while peanuts accounted for 25% of the diet, although when pine seed was scarce, peanut consumption could rise to make up 57% of the diet (Shuttleworth, 1997). Peanuts

have a high fat content and contain phosphorous and enzymes that inhibit amino acid absorption, which limits how much an individual animal can consume (Shuttleworth, 1997). In studies of food preference, peanuts have been shown to be less preferred than other deciduous nuts, which may be linked to their lower nutritional value (Kostrzewa & Krauze-Gryz, 2020).

Formby is an extreme example with one type of supplementary food being provided as a conservation tactic, but other populations receive more casual supplementary feeding, particularly in suburban or urban areas where food is provided in gardens and parks. Jersey is largely suburban or farmland but there are some woodlands of oak, sweet chestnut and Scots pine (Magris & Gurnell, 2002). However, squirrels are found across the entire island (Simpson et al., 2013), making use of supplementary food left out by the public (Magris & Gurnell, 2002). The Isle of Wight also has a thriving population of red squirrels despite much of the island being denuded of woodland, with The Isle of Wight Red Squirrel Trust noting that there are few areas where supplementary feeding is not available to squirrels (Butler, 2021). The specifics of the food items provided to red squirrels is not recorded but likely to consist of commercial food items and seed mixes typically sold in garden centres and pet stores. A comprehensive list of the diet of red squirrels in Britain is included in Table 1.1.

1.4.4 Caching

Which food items are regularly consumed throughout the year is variable. The fruiting period of tree species occurs at different times throughout the year. The abundance of the annual crop can also vary greatly between years. Conifer seeds from Scots pine and Sitka spruce are consumed throughout most of the year but mainly in spring and summer. Hazelnuts, beechnuts, and acorns are consumed mostly in autumn (Moller, 1983). Stomach content analysis shows that in Autumn months more fungi is consumed than in any other season (Robb et al., 2008a). This variability of access to food items is remedied through caching behaviours. Squirrels will store surplus food for later consumption (Starkey & del Barco-Trillo, 2019). Red squirrels cache food through scatterhoarding, where food is scattered widely across the home range in small caches of only one or two food items per cache (Shuttleworth, 2000; Wauters et al., 1995). Food is generally cached in the autumn and recovered throughout winter and spring (Wauters et al., 1995). This energy reserve is particularly important in spring when energy costs are high, as females are providing milk for litters and males are taking part in mating-chases (Wauters et al., 1995).

Table 2.1. List of British Red Squirrel Populations (as of 2023) including the woodland/habitat, probable diet and genetic background of those populations.

Location	Habitat Type	Probable Diet	Genetic Background
Scottish Highlands and Aberdeenshire	Mostly of native Scots pine <i>Pinus sylvestris</i> , with some timber plantations bringing in non-native conifer species (Moller, 1986; Tittensor, 1970)	Mostly Scots pine seed and non-native spruce seed. (Kenward & Holm, 1993)	Possibly native, some recent Scandinavian introductions (Barratt et al., 1999; Harvie-Brown, 1880; Lurz, 2010)
South Scotland	Non-native conifers of Kielder Forest (along Anglo-Scottish border) (Hale & Lurz, 2003).	Conifer seed (assumed from available habitat type)	Likely mix of native squirrels with recent Scandinavian genetic background (Hale et al., 2001, 2004)
Cumbria	Pockets of mixed woodland with hazel <i>Corylus avellana</i> , oak <i>Quercus sp</i> and yew <i>Taxus baccata</i> but some areas dominant in non-native conifer species (Hale & Lurz, 2003; Tonkin, 1983),.	Sitka spruce, Norway spruce, lodgepole pine seed (Gurnell et al., 2004b), Hazelnuts, yew seeds (Tonkin, 1983)	Likely mix of native squirrels with recent Scandinavian genetic background (Hale et al., 2001, 2004)

Northumberland	Non-native timber plantations of Sitka spruce <i>Picea sitchensis</i> , Norway spruce <i>Picea abies</i> and lodgepole pine <i>Pinus Contorta</i> (Hale & Lurz, 2003)	Conifer seed (assumed from available habitat type) Beech Seed (Shorten, 1954)	Likely mix of native squirrels with recent Scandinavian genetic background (Hale et al., 2001, 2004)
Formby & Sefton Coast	Scots pine <i>Pinus sylvestris</i> with some Corsican pine <i>Pinus nigra</i> and lodgepole pine <i>Pinus contorta</i> (Shuttleworth, 2000)	Conifer seed and peanuts (Shuttleworth, 1997)	European ancestry (Gurnell & Pepper, 1993)
Brownsea Island	Scots pine and maritime pine <i>Pinus pinaster</i> (Thain & Hodder, 2015)	Conifer seed (assumed from available habitat type)	Remnant native populations but dispersal from Furzey island may be the source of shared haplotypes between the two islands (Hardouin et al., 2019; Thain & Hodder, 2015).
Furzey Island	Mature Scots pine, corsican pine, peripheral oak and sweet (Kenward & Hodder, 1998)	Scots pine and Corsican pine seed (Kenward & Hodder, 1998)	Introduced from Northern England in 1970's (Kenward & Hodder, 1998)

Isle of Wight	Oak-hazel (Kenward & Holm, 1993) Suburban and farmland(Butler, 2021).	Hazelnuts and acorns. ((Kenward & Holm, 1993) Supplementary food provided by people (Butler, 2021).	Introduction to the island is not documented but genetic analysis suggests their source population is from southern English populations, now extinct (Hardouin et al., 2019).
Wales (Not including Anglesey)	Non-native timber plantations (Sitka spruce, Norway spruce and larch.	Conifer seed and supplementary foods (Shuttleworth, 2015)	Likely native to Wales (Hobbs, 2005; Ogden et al., 2005)
Anglesey	Mixed spruce and Scots pine (Mason & Young, 2021; Shuttleworth, 2015)	Conifer seed and some supplementary foods (Shuttleworth, 2015)	Likely native to Wales (Hobbs, 2005; Ogden et al., 2005)
Jersey	Oak and sweet chestnut with some Scots pine (Magris & Gurnell, 2002)	Oak, sweet chestnut, conifer seed and supplementary food (Magris & Gurnell, 2002)	Introduced from both Southern England and mainland Europe (Le Sueur, 1976; Simpson et al., 2013)
Thetford	Scots and Corsican pine, oak and beech (Gurnell et al., 1997)	Conifer seed (Gurnell et al., 1997)	Remnant Native Population

1.5. Morphology of the Masticatory Apparatus

1.5.1 Muscles of Mastication

In mammals, mastication involves three distinct muscle groups: the temporalis muscle, the masseter muscles, and the pterygoid muscles. In some herbivores, the masseter is extended further than in carnivores, allowing for exertion of high masticatory pressure at the molar teeth, an adaptation for grinding of tough plant material (Turnbull, 1970). In rodents, this configuration is more extreme, with the masseter muscle the dominant muscle of mastication. It is located more anteriorly on the jaw, allowing for strong forces to be generated at both the incisors and molars (Cox & Jeffery, 2011). The relative positions and shape of the masseter muscle is used to describe three distinct morphologies in the rodents. First formally described by Brandt (1855), and formalised by Wood (1965), these are held today as the Sciuromorpha, Hystricomorpha and Myomorpha. Additionally, there is a fourth recognized category – Protrogomorpha, considered the primitive condition, and is only seen in one extant species, the mountain beaver *Aplodontia rufa* (Druzinsky, 2010a). Historically these morphologies were used as the basis of division of rodents into sub-orders, but genetic analysis has not supported monophyly of these groups, instead an example of convergent evolution (Renaud & Michaux, 2003; Swanson et al., 2019). Sciurids (the squirrel family) are some of the best examples of sciuromorphs but their closest relatives, the Gliridae (dormice), do not have a sciuromorphic masticatory system (Adkins et al., 2003; Blanga-Kanfi et al., 2009; Huchon et al., 2002; Swanson et al., 2019). Confusion can arise as some studies still use the term ‘sciuromorpha’ to refer to the phylogenetic group as opposed to the morphology (e.g. Swanson et al. 2019). Nomenclature of the rodent masticatory apparatus is also inconsistent across studies, with differing numbers of muscles identified with different names (Cox & Jeffery, 2011). Here I use muscle nomenclature proposed by Cox & Jeffery (2011), and refer to sciuromorphy as the morphological condition only.

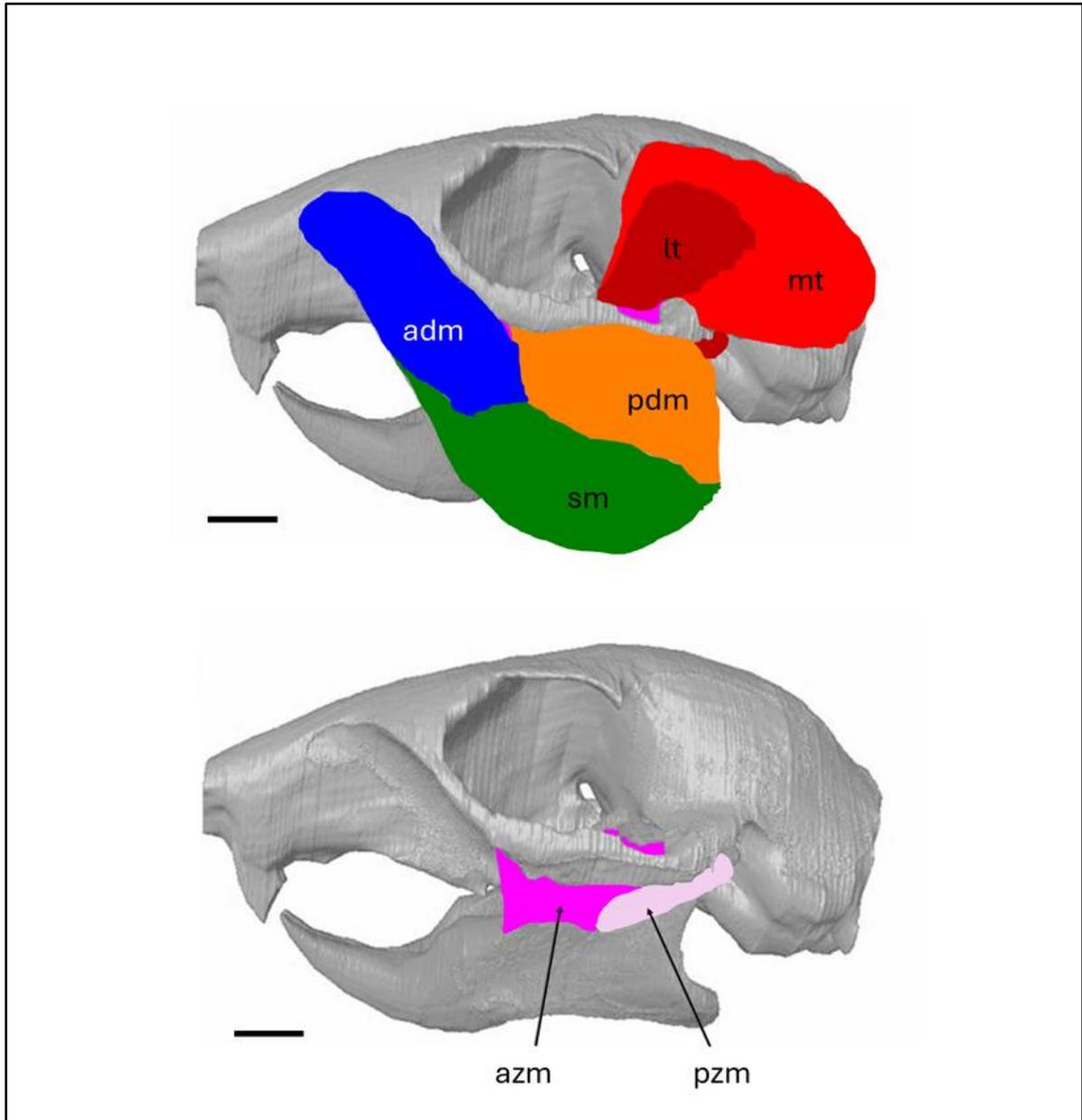


Figure 1.1 Models of squirrel crania with muscles of mastication labelled, adapted from Cox & Jeffery (2011). adm, anterior deep masseter; mt, medial temporalis; pdm, posterior deep masseter; lt, lateral temporalis; sm; superficial masseter; azm, anterior zygomaticomandibularis; pzm, posterior zygomaticomandibularis. All scale bars are 5 mm.

1.5.2. Masseter Muscle

In Sciuriforms, the masseter muscle extends underneath the zygomatic arch onto the rostrum. This structure is thought to give sciuriforms increased gnawing efficiency (Cox et al., 2012). It is divided into three layers: the superficial masseter, the deep masseter and the zygomaticomandibularis (Becht, 1953; Cox & Jeffery, 2011).

The superficial masseter is the largest layer. It is the main protractor of the jaw, while also playing a role in jaw closing and power strokes of both gnawing and chewing. In sciuriforms, the margin of the superficial masseter is diagonal across the zygomatic arch, revealing the deep masseter underneath. The pars reflexa, the reflected part of the superficial masseter around the ventral margin of the lower jaw, is smaller than other rodents, as they have a large medial pterygoid muscle (Cox & Jeffery, 2015). The superficial masseter originates just below the infraorbital foramen, from a flattened tendon attached to small process and inserts in two locations on the mandible. The postero-dorsal muscles fascicles insert on the posterolateral surface of the angle of the jaw ramus and the anteroventral fascicles insert on the medial surface of the jaw, just ventral to the insertion of the internal pterygoid.

The deep masseter is sandwiched between the superficial masseter and zygomaticomandibularis. Originating from the ventrolateral surface of the zygomatic arch and inserting on the lateral surface of the mandible, along the masseteric ridge. The deep masseter forms over 30% of the masticatory muscle volume. In sciuriforms, the deep masseter is anteriorly expanded onto the rostrum, originating from the masseteric fossa. This increases the relative length of the moment arm and significantly contributes to the strength of the bite force that can be produced at the incisors (Casanovas-Vilar & van Dam, 2013). In squirrels, the deep masseter is divided into anterior and posterior parts. The innermost layer, the zygomaticomandibularis, in contrast makes up less than 10% of the masticatory musculature. In squirrels, it originates on the medial surface of the zygomatic arch and inserts in the masseteric fossa, just posterior to the toothrow.

1.5.3 Temporalis Muscle

The temporalis muscle is relatively small, forming around 10% of masticatory muscle mass. There is some division of this muscle into medial and lateral parts. The medial temporalis has a wide origin on the cranium that converges to a small region on the medial surface of the mandible, between the coronoid process and the retromolar fossa. The lateral fibres originate from the anterior half of the fascia overlying the medial temporalis and insert on the coronoid process. The temporalis functions most effectively as the power stroke of mastication. The division into medial and lateral parts has been proposed to allow operation in both gnawing and chewing; the lateral temporalis stabilizing and elevating the mandible for incisor biting while the medial is more responsible for molar grinding (Hiimae & Ardran, 1968).

5.1.4 Pterygoid Muscles

In rodents, there are usually two pterygoid muscles – internal and external. In squirrels, the internal pterygoid originates both in the pterygoid fossa, posterior to the molar tooth row and on the lateral surface of the pterygoid process. It has a wide insertion point on the medial surface of the angular process. In sciuriforms, it is thought to function as a counterbalance to the dorso-lateral pull of the deep masseter and reducing overall mandibular stress (Cox & Jeffery, 2015). The external pterygoid is the smallest of the masticatory muscles (3-4% of masticatory muscle mass), originating along the ventral margin of the skull and inserting on the medial condyloid process, just below the condyle (Cox & Jeffery, 2015; Cox & Jeffery, 2011). Contraction of the internal pterygoid produces lateral movement (Hiimae & Kay, 1972).

5.1.5 Skeletal Anatomy and Dentition

Muscles involved in biting and mastication have attachment points on the coronoid and angular processes of the mandible, with the mandibular condylar process pivotal in the articulation of the skull (Doudna & Danielson, 2015).

Like all rodents, red squirrels have paired incisors that grow continuously throughout their life. Sharpness is maintained through differing wear rates between the mesial surface of the incisor (which has a thick layer of enamel) and the distal surface which has no enamel (Shorten, 1954). Incisors are used for gnawing, a dorso-ventral slicing or scraping motion that is effective for accessing some of the common foods consumed by squirrels. There are four pairs of grinding cheek teeth (PM3-M3). Rodents have distinctive dentition with a large diastema separating the paired incisors from the cheek teeth. These two dental regions have separate functions which are mutually exclusive as the incisors cannot be in occlusion at the same time as the molars (Anderson et al., 2014; Michaux et al., 2007; Renaud & Auffray, 2010). Rodents are capable of propalinal movement of the lower jaw to switch between functions. Pre-oral processing, in the form of ‘gnawing’ requires a protrusion of the jaw to bring the incisors into occlusion. In chewing, the jaw is retracted to bring the cheek teeth into occlusion (Anderson et al., 2014). Thus, the maximum bite force exerted by a rodent can only be placed at either the incisors or molars asynchronously. Presumably, the highest bite forces must be exerted at the incisors in order to ‘break’ food items for further mastication. Bite force (the force in newtons that an animal can exert at the biting tooth) is a parameter often measured to better understand the ecology of both extant and extinct animals (e.g Rayfield,

2004; Wroe et al., 2005). Bite force can be measured with in vivo experiments using force transducers (e.g. Herrel et al., 1999; Van Daele et al., 2009; Becerra et al., 2014) or can be calculated through modelling based on cross-sectional areas of the relevant muscles and measurements of the muscle lever-arms (Blanco et al., 2012; Cox et al., 2012; Cox & Jeffery, 2015; Druzinsky, 2010b).

The tooth morphology is no doubt highly influenced by the food items consumed by squirrels. Primarily granivores, squirrels main difficulty is biting through the outer shells of edible seeds. This dentition allows concentration of force into incisor biting.

1.5.6 Evolution and Plasticity of the Masticatory Apparatus

Mandible shape is highly conserved in squirrels (Anderson et al., 2014; Atchley et al., 1992; Mavropoulos et al., 2005; Mezey et al., 2000), but changes can occur in the mandible through evolutionary selection and plasticity. The fast generation time in rodents allows them to be excellent organisms to study evolutionary change. Studies have shown that in mice, evolutionary change in the mandible can occur in just 100 years (Mezey et al., 2000).

Phenotypic plasticity of skeletal tissue also allows for the change of mandible shape within the lifetime of an animal. This can occur via bone modelling (growth of the bone through ontogeny) and bone remodelling (bone changing shape within the life span of the animal). The mandible is responsive to differences in diets, with phenotypic plasticity shown to occur in mice when fed diets of different levels of 'hardness' (Anderson et al., 2014). Where mice consumed a 'soft' diet for a significant period of time, their mandibles showed shape changes that resulted in a lower mechanical advantage compared to those mice consuming a 'hard' diet. These changes occurred within the life span of individual mice, therefore the shape change was a result of bone remodelling (Anderson et al., 2014). This study also explored the modularity of the mandible, showing that the mandible has different functional modules within developmental ones (Anderson et al., 2014). The ascending ramus and alveolar region of the mandible are functional units and are independent of genetic structure (Mezey et al., 2000). The mandible functional apparatus (e.g. the adductor muscles, jaw joints, the incisors and molar teeth) all act on the bone in a localised manner. Integration between these functional modules was shown to be higher in mice fed a hard-food diet when compared with those fed a soft-food diet.

In British red squirrel populations, the habitats that different populations occupy offer different food items for consumption by squirrels. Some populations likely regularly consume

a diet of relatively ‘harder’ food items than others. With combined genetic isolation of these populations and them experiencing different selection pressures, there is potential for morpho-functional specialisations arising as the masticatory apparatus adapts to a particular diet. Either evolutionarily, or through adaptive plasticity, variation in morphology will likely have functional consequences. Indeed, some biomechanical variation in British red squirrel populations has already been detected (Cox et al., 2020). As red squirrels are an endangered species in Britain, how these populations are surviving and adapting is of importance. Particularly when considering foods that are available/provided to populations. As previously discussed there are many potential negative effects of supplementary feeding. A little explored area is how animals may become over reliant on supplementary foods to the point of adapting to consume mostly that diet.

1.6. Statement of Aims

The aim of this thesis is to investigate the effect of population fragmentation, isolation, variation in diet and supplementary feeding on the masticatory apparatus of British red squirrels across their different populations. Through three research chapters, the morphological variations, mechanical challenges of the diet and the functional consequences of morphological change will be investigated, as follows:

Chapter 2 - Assessing Morphological Variation, Spatially and Temporally, in British Red Squirrel Populations

This chapter aims to assess the skeletal morphology of the crania and mandible of British specimens of the Eurasian red squirrel, *Sciurus vulgaris*, in order to further observe, both spatially and temporally, predicted morphological variation in the masticatory apparatus between populations, as first noticed by (Cox et al., 2020). To achieve this, pre-prepared skeletal specimens from the National Museum of Scotland will be imaged, digitised, and analysed for variation in shape and size through statistical methods, namely geometric morphometrics.

Chapter 3 - Investigation of the Material Properties of Red Squirrel Food items and the effect of caching.

This chapter aims to link the morpho-functional variation in the red squirrel mandible to the diet consumed by distinct red squirrel populations within Britain. To achieve this, a self-devised method of assessing material properties of food items, namely toughness, will be

employed and representative food items consumed by the main populations of British red squirrels will be analysed.

Chapter 4 - Investigation into Biomechanical Function of the Red Squirrel Mandible using Finite Element Analysis

This chapter aims to link the results of Chapter 2 to the results of Chapter 3 by assessing the functional abilities (biomechanical performance) conferred by the differing morphologies of British red squirrel mandibles and their relative abilities to consume different diets. To achieve this, skeletal specimens representative of different populations will be microCT scanned, converted to models and analysed through finite element analysis.

2. Assessing Morphological Variation, Spatially and Temporally, in British Red Squirrel Populations

2.1 Introduction

Isolated populations of mammals, such as those on islands, exhibit high rates of morphological evolution (Millien, 2011). It has been shown that on smaller islands with smaller populations, mammals evolve more rapidly and to a greater extent than mainland populations (Millien, 2011). Fragmented populations, caused by habitat loss or other ecological barriers, will experience similar levels of genetic insularity. These isolated populations are acted on by the same evolutionary processes as island populations (Pergams & Lawler, 2009) and can lead to similar rates of phenotypic change. Case studies of island evolution (functional or geographical) often involve rodents, as they have short generation times and are widely documented in museum collections (Holmes et al., 2016). Rodents are also highly adaptable through morphological change (Auffray et al., 2009), either within the lifetime of the animal (plasticity i.e. through bone remodelling) or microevolution (Stumpp et al., 2018). The masticatory apparatus of lab mice, for example, has been shown to rapidly respond in shape to the mechanical properties of diet (Mezey et al., 2000; Anderson et al., 2014) demonstrating how phenotypic changes (mandible morphology) can be linked to a functional change (mastication). Of course, when looking to attribute morphology to adaptive responses, consideration must be given to the genetic ancestry of populations, as factors such as the founder effect or genetic drift can influence phenotypic characteristics of a population. Nonetheless, researchers have argued that selection can act quickly on isolated populations of rodents, and is often responsible for shifts in morphology (Pergams et al., 2015).

British populations of red squirrels (*Sciurus vulgaris*) are an ideal case-study for investigating the effects of isolation on the morphology of fragmented populations. Prior to the introduction of the invasive grey squirrel (*Sciurus carolinensis*), red squirrels were the only species of squirrel in Britain. Similar to other areas where grey squirrels have been introduced, namely Ireland (Watt, 1923) and parts of Northern Italy (Currado & Scaramouino, 1989; Currado et al., 1987; Gurnell & Pepper, 1993), red squirrel populations have suffered a significant decline in numbers as a result of their congener's arrival (Ó'Teangana et al., 2000; Shorten, 1952; Signorile et al., 2014). Grey squirrels were first introduced to England in 1890 and have proliferated greatly since then (Middleton, 1930; Shorten, 1952). Much research has been centred on the success of grey squirrels in Britain

and the concurrent decline of red squirrels, but it was only within the last few decades that the squirrel-pox virus (SPQV), a relatively harmless disease for grey squirrels, but largely fatal for reds, was discovered as a major factor (Sainsbury & Gurnell, 1995; Sainsbury & Ward, 1996; Rushton et al., 2000, 2006; Tompkins et al., 2003; Chantry et al., 2014). In areas where grey squirrels are present, reds suffer almost immediate catastrophic declines due to outbreaks of SPQV (Tompkins et al., 2002; Chantry et al., 2014). This has led to a highly fragmented distribution of red squirrels, remaining in strongholds that usually have physical barriers to grey squirrel encroachment (i.e. islands such as Jersey, Brownsea, Isle of Wight and Anglesey) or where the habitat is better suited to the diet of red squirrels (pine forests of Northern Scotland)(Crawley, 2020). Some scattered populations remain in woodland jointly with greys but only where significant grey squirrel eradication programmes are in place (Shuttleworth et al., 2021). Eradication programmes are one method employed by conservation bodies to boost red squirrel numbers, with translocation also used. This has led to a muddy knowledge of the genetic history of red squirrels in Britain, as many translocations are not well documented (Kenward & Holm, 1989; Hale et al., 2004).

The sub-populations of red squirrels in Britain occupy a variety of woodlands offering different food sources. Pine seeds from Scots Pine, *Pinus sylvestris*, make up a large proportion of the diet of red squirrels living in pine forests (Moller, 1986; Shuttleworth, 2000), but also seeds from non-native conifers such as Norway Spruce *Picea abies* and Sitka Spruce *Picea sitchensis* (Moller, 1986; Hale & Lurz, 2003; Shuttleworth, 2000; Gurnell et al., 2004) which are commonly grown as timber plantations in the UK (mainly in North Scotland but also in some parts of Northern England e.g. Kielder forest). Squirrels in deciduous or mixed woodland, common in the Lake District and the Isle of Wight, may have a diet rich in hazelnuts *Corylus avellana*, acorns *Quercus sp* and beechnuts *Fagus sylvatica* (Tonkin, 1983; Moller, 1983, 1986; Magris & Gurnell, 2002). Additionally, populations in urban areas or in national parks may receive quantities of supplementary food provided by the general public or conservation groups (e.g. Formby National Reserve, Merseyside). Formby hosts a relatively large population of red squirrels where, up until recently, conservation workers supplemented the squirrels' diet with peanuts (Shuttleworth, 2000).

Squirrels are adept at accessing the hard, outer casing of seeds such as acorns, or dismantling cones to access seeds. In such cases, being able to generate higher bite forces can be a competitive advantage (Young et al., 2007). The main constraint for bite force is skull morphology (Swartz et al., 2003; Wroe & Milne, 2007; Anderson & LaBarbera, 2008;

Nogueira et al., 2009; Maestri et al., 2016;), as cranial and mandibular morphology influences the size and orientation of the muscles of mastication. Anatomical studies have shown that cranial and mandibular morphology can experience change in response to dietary selection pressures (Christiansen & Wroe, 2007; Wroe & Milne, 2007; Anderson & LaBarbera, 2008; Nogueira et al., 2009; Maestri et al., 2016) which then has an influence on bite force. Considering this high adaptability of the rodent mandible, it is therefore expected that squirrels consuming particular diets will be functionally adapted to the physical challenges of accessing that food item.

Bite force

Previous work has suggested that this may be the case in British populations where the mechanical advantage of the temporalis muscle, an important muscle in mastication, is lower in squirrels from Formby, than other populations (Cox et al., 2020). This has been hypothesized as an adaptive response to a diet rich in peanuts. Bearing in mind the different diets of British red squirrel populations, and their degree of isolation from each other, it is reasonable to assume there will be differing cranial, as well as mandibular, morphology between these populations.

2.1.1 Aims

This study aims to quantify the morphological variation of the crania and mandible between populations of the Eurasian red squirrel, *Sciurus vulgaris*, in Britain, both spatially and temporally. To achieve this, pre-prepared skeletal specimens from the National Museum of Scotland were imaged, digitised, and analysed for variation in shape and size through statistical methods, namely geometric morphometrics.

2.1.2 Hypotheses

1) *Extant populations of Eurasian red squirrels in Britain are statistically different from one another in cranial and mandibular morphology.* This is predicted as populations of red squirrels in Britain are fragmented and isolated from each other, and occupy different climatic conditions and habitat types. Previous work has shown that the masticatory apparatus is highly responsive to the mechanical properties of food types (Anderson et al., 2014). As different food types are available in the different habitats of British populations, it is expected that different populations will show morphological variation that has arisen for different mechanical functions to deal with particular food types, either through adaptation or

plasticity. This will be tested through geometric morphometric analysis and comparative statistics.

2) *Statistically significant morphological change over time can be detected in the crania and mandible of British red squirrel populations.* This is predicted as populations that have been isolated for a longer period of time are more likely to exhibit morphologies less similar to each other, as they become more adapted to their particular habitat while limited exchange occurs with other populations. This will be tested through comparative statistical methods and geometric morphometrics, comparing shape and size between past and present populations, and particular focus on within population changes over time.

3) *Morphological variation between populations will result in differences in mechanical advantage of the muscles of mastication.* This is predicted as differences in morphology will likely affect the ratio of muscle in-lever to biting out-lever. This will be analysed through calculations of simple linear measurements of the mandible and populations will be compared through ANOVA.

2.2 Materials and Methods

161 cranial and 388 mandibular specimens of red squirrels from the UK were included in the dataset. Specimens were prepared by the National Museum of Scotland and collected along with data detailing the source population, sex and year collected of the specimens (see Appendix 1 for details related to each specimen used in this analysis). All specimens were opportunistic collections of deceased squirrels that had died of natural causes or in road traffic accidents. Collection locations were grouped into larger populations where dispersal of individual squirrels remains possible. These were Northern Scotland (North of the Central Belt), Formby (National Trust reserve of at Formby and surrounding areas of Lancashire), and Jersey (Channel Islands) and a fourth populations which grouped together Southern Scotland (south of the Central belt) and Northern England (Cumbria, Northumberland, Durham and Yorkshire Dales) and will be referred to here as the Borders population (see Figure 2.1). Collection dates were organised into intervals of 10 years between 1960 and 2010 (see Table 2.2).

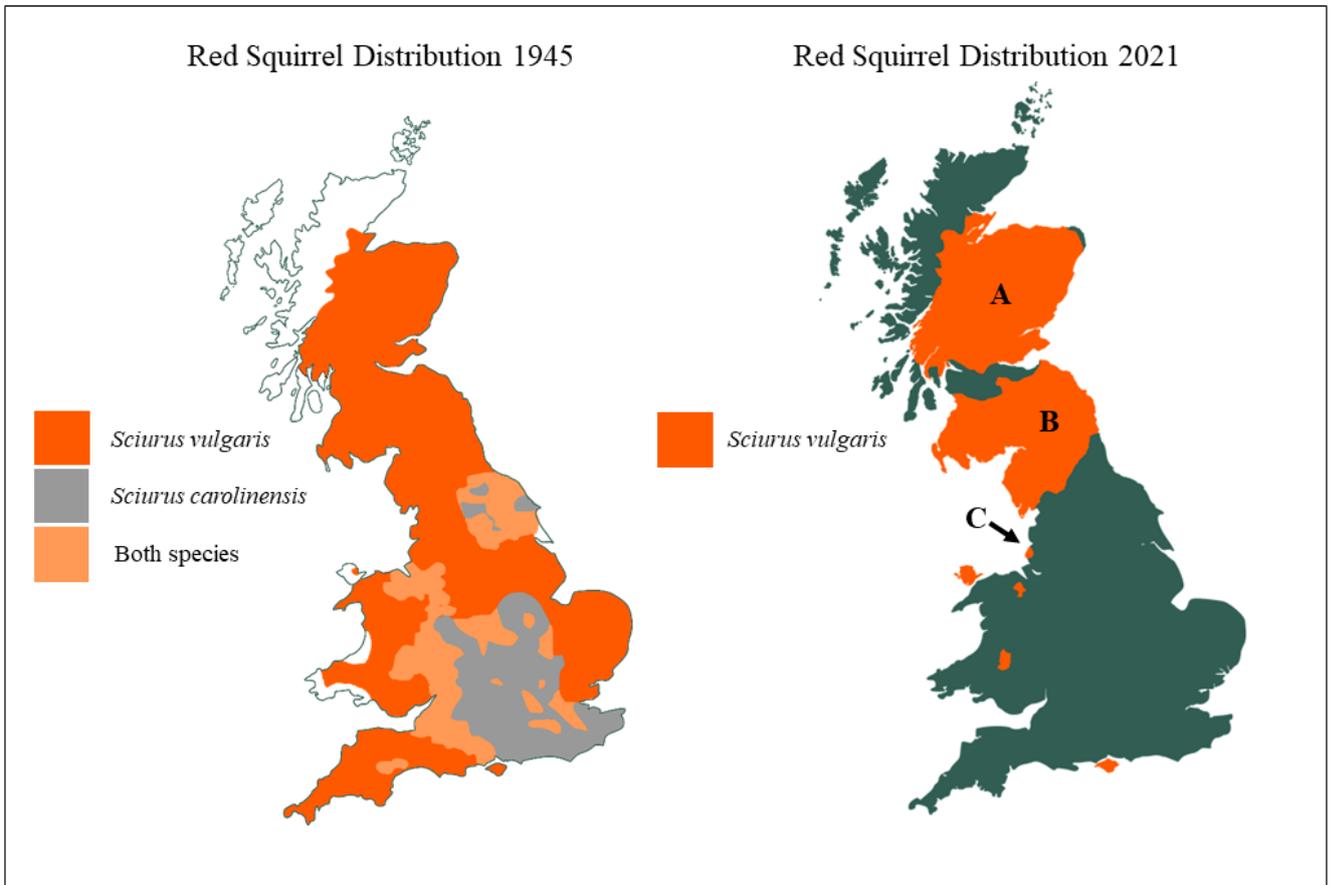


Figure 2.1. Maps of Britain showing distribution of red squirrel *Sciurus vulgaris* in 1945 (adapted from Matthews et al., 2018) and 2021 (adapted from Cox et al. 2020). 2021 map shows populations included in this study, but excludes some smaller populations such as Brownsea Island and Jersey. Labelled populations are (A) North Scotland, (B) Borders and (C) Formby.

Table 2.3. Number of Specimens from each location and decade, where decades are divided as: specimens grouped into “1990” were collected between 1990 – 1999 inclusive, except for those in 2010 where specimens collected in 2020 are also included.

Decade	Sex	North Scotland		Borders		Formby		Jersey	
		Crania	Mandibles	Crania	Mandibles	Crania	Mandibles	Crania	Mandibles
1960	Female	1	3						
	Male	6	8	2					
1970	Female		1						
	Male								
1980	Female		3	2	3				
	Male	3	5						
1990	Female	12	27	18	33	11	21		16
	Male	9	30	15	39	13	31		11
	Unknown	1		2	5		1		
2000	Female	5	6	3	5				6
	Male	5	8		7				4
	Unknown		1						
2010	Female					32	47		
	Male		1			32	58		
	Unknown					1			
Total		44	92	44	92	89	164		37

2.2.1 Landmarking

Crania were digitized using an Artec Space Spider (Artec 3D, Luxembourg), a high-resolution 3D surface scanner. This scanner makes use of structured blue-light scanning, which works by projecting a known pattern onto an unknown surface and analysing the deformation in the surface to reconstruct the shape of the object. Point clouds (series of points in 3D space) are generated which represent the unknown surface. Using associated Artec software (Artec Studio v15), point clouds can be converted to a meshed surface (surfaces are created between 3 connected points) and high-resolution colour data can be projected onto each surface to create a highly accurate 3D model. Specimens were scanned and saved as STL files. 3D landmarks were recorded on the virtual reconstruction using Avizo 9.2 Lite (Thermo Fisher Scientific, Waltham, MA, USA). 21 landmarks were placed on the left side of the crania only (see Figure 2.2 and Table 2.3 for full list of anatomical positions of landmarks). Four surface paths were also placed in Avizo and converted to semi-landmarks in R (R Core Team, 2017) using the Arothron package (Profico et al., 2021). Semi-landmarks

along the surface paths that overlapped with other landmarks were removed in R, for inclusion in the overall landmarks of the crania (see Figure 2.3). Cranial landmarks and semi-landmarks were adapted from previous studies on various rodent crania (Morris et al., 2018; Myers et al., 1996; Swiderski et al., 2000). Specimens where all landmarks could not be placed due to broken crania were removed from the “whole crania” subsample. However, if some curves could be landmarked, these were saved separately to be analysed later as a separate sample. This process resulted in six subsamples with differing numbers of specimens; “Whole Crania”, “Dorsal Midline”, “Orbit”, “Temporalis Scar” and “Zygomatic Arch”.

Hemi-mandibles of red squirrels are almost completely flat; therefore, it was deemed appropriate to use 2D images. 105 hemimandibles were photographed by me, using a Nikon 32000 DSLR camera, taken of the medial side of the hemimandible which were placed against a black background with a scale bar included. 388 photographs of hemimandibles that were originally taken by Cox et al. (2020) were taken on a Panasonic Lumix DMC-TZ60, against gridded paper of 0.5cm squares for scaling. Both sets of photos were landmarked. Landmarks were placed with tpsdig (Rohlf, 2023). 13 landmarks, based on those from Cox et al. 2020, were placed at anatomical locations chosen to provide thorough coverage of the hemi mandible (see Figure 2.4), based on previous studies on the rodent mandible (Casanovas-Vilar & van Dam, 2013; Cox et al., 2020; Zelditch et al., 2008, 2015). Additionally, 5 curves were drawn along the perimeter of the mandible and semi-landmarks were placed along these curves equidistantly. Curves were appended to the landmarks in tpsUtil. Where points along the curves overlapped with other landmarks, these points were removed in tpsUtil. In total 82 mandible landmarks were included (see Fig 2.4 and Table 2.4).

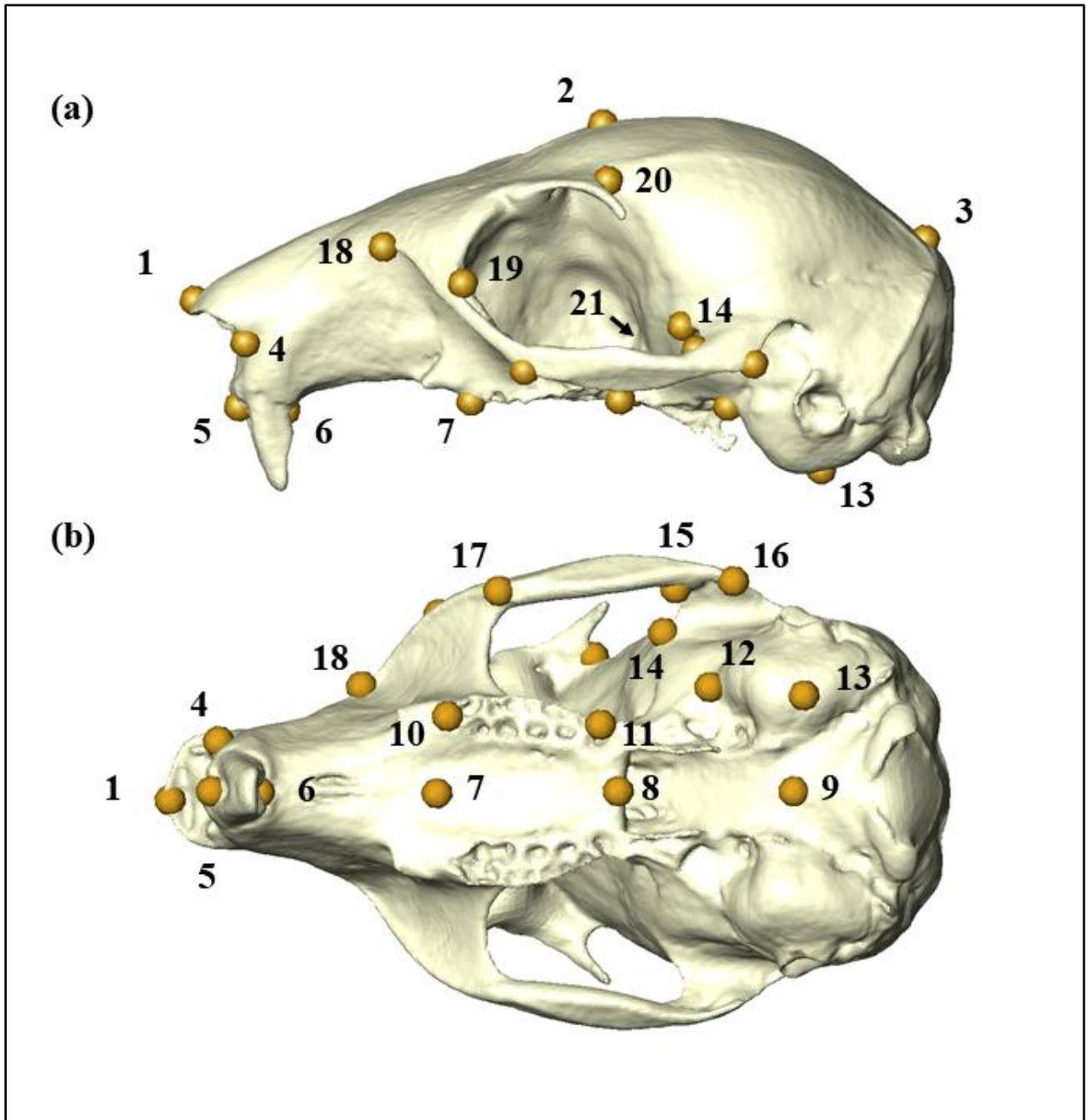


Figure 2.2 Landmark configuration on red squirrel crania showing (a) Left lateral view and (b) ventral view. Landmark descriptions given in Table 2.3. Specimen NMS GH166.09

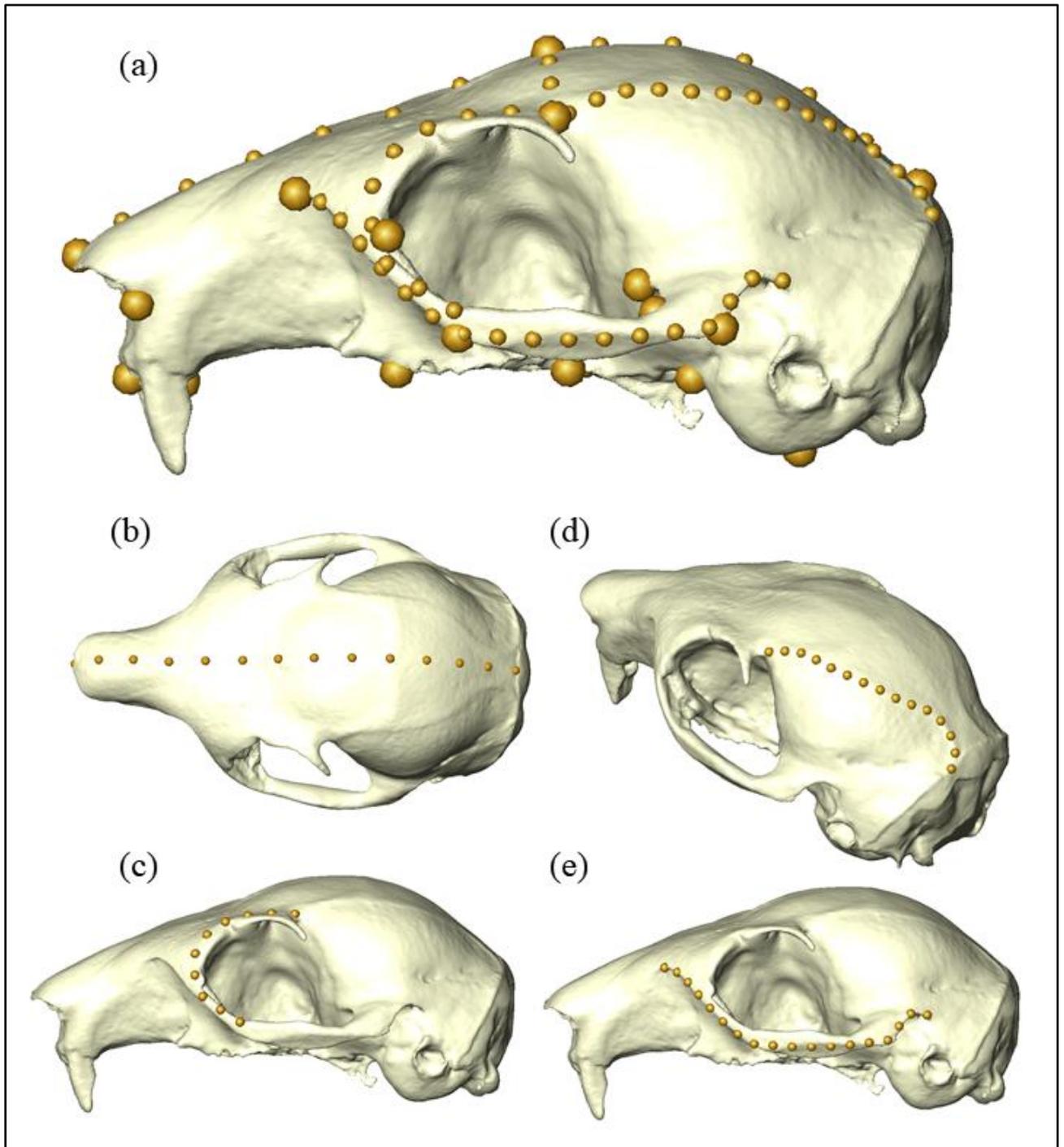


Figure 2.3. Landmark configuration (a) Lateral view of full cranial landmarks with semi-landmarks, and semi-landmarks along five curves (b) Dorsal midline (c) Orbit (d) Temporalis Scar and (e) Zygomatic Arch. Landmark descriptions given in Table 2.3. Specimen NMS GH166.09.

Table 2.3. Morphological descriptions of cranial landmarks and curves.

Cranial Landmarks		
LM 1	Anterior-most point on rostrum/internasal suture	
LM 2	Midpoint of line running between dorsal orbital notches	
LM 3	Posteriormost point on dorsal midline	
LM 4	Anteriormost point on naso-premaxillary suture	
LM 5	Anterior midpoint of incisor alveoli	
LM 6	Posterior midpoint of incisor alveoli	
LM 7	Midpoint between anteriormost points of first premolar alveoli	
LM 8	Posteriormost midline point on palate	
LM 9	Midline point of the ventral margin of the foramen magnum	
LM 10	Anteriormost point on alveolar margin of first premolar	
LM 11	Posteriormost point on alveolar margin of third molar	
LM 12	Anteriormost point of auditory bulla	
LM 13	Ventral apex of auditory bulla	
LM 14	Anterior extremity of zygomatic process of squamosal	
LM 15	Posteriormost point on anterior margin of zygomatic process of squamosal	
LM 16	Posteriormost point on jugal bone	
LM 17	Postero-lateral extremity of zygomatic plate	
LM 18	Anteriormost extremity of zygomatic plate	
LM 19	Ventralmost point of lacrimal tubercle on orbital margin	
LM 20	Posterior junction of postorbital process and cranial vault (dorsal orbital notch)	
LM 21	Anteriormost point on margin of optic foramen	
Cranial Curves		Number of Semi-Landmarks
Dorsal midsagittal line of cranium (LM1 to LM3)		11
Anterior orbital margin (LM20 to LM17)		8
Ventral margin of zygomatic arch (from C18 to posterior extremity of zygomatic process of squamosal).		17
Temporal ridge from C20 to nuchal line..		14

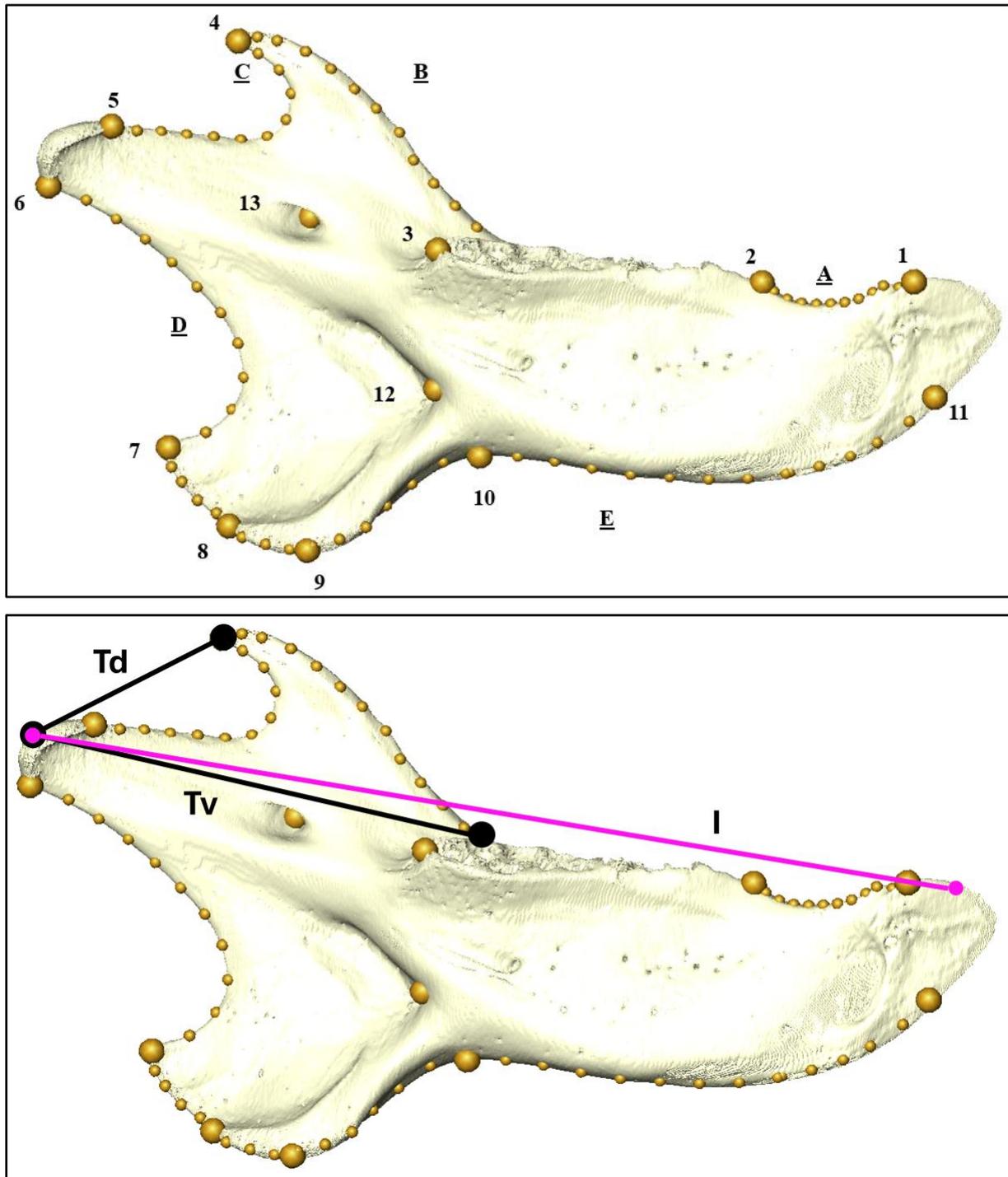


Figure 2.4. Medial views of the hemimandible of Specimen NMS SR106.20 showing landmarks 1-13, curves A-E (placed in tpsDig) and measurements taken for mechanical advantage calculations. Landmark descriptions are given in Table 2.4. Measurements are of two in-levers: Td (the most dorsally inserting fibres of the temporalis muscle) and Tv (the most ventrally inserting fibres of the temporalis muscle) and the out-lever: I (distance between the condylar process and where the incisor tooth erupts from the mandible).

Table 2.4. Morphological descriptions of mandibular landmarks and curves

Mandibular Landmarks		
LM1	Dorsalmost point on margin of incisor alveolus	
LM 2	Mesial margin of alveolus of first premolar	
LM 3	Distal margin of alveolus of third molar	
LM 4	Tip of coronoid process	
LM 5	Rostral margin of mandibular condyle	
LM 6	Caudal margin of mandibular condyle	
LM 7	Caudal tip of angular process	
LM 8	Ventralmost point of pterygoid fossa on margin of angular process	
LM 9	Ventralmost point on margin of angular process	
LM 10	Dorsal point of inflection between angular process and mandibular body	
LM 11	Ventralmost point on the incisor alveolar margin	
LM 12	Rostralmost point of pterygoid fossa	
LM 13	Centre of mandibular foramen	
Mandibular Curves		Number of Semi-Landmarks
A	Margin of diastema between M1 and M2	10
B	Anterior margin of coronoid process between second molar and M4	10
C	Condylar notch between M4 and M5	11
D	Posterior margin of mandible between M6 and M7	10
E	Ventral margin of mandible between M7 and M11	28

2.2.2 Statistical Analysis

Initial exploratory analysis was performed using MorphoJ (Klingenberg, 2011) and further analyses were performed in R using the packages Arothron (Profico et al., 2021), Morpho (Schlager, 2017), geomorph (Adams et al., 2021; Baken et al., 2021), RRPP (Collyer & Adams, 2018, 2021), vegan (Oksanen et al., 2020) and RVAideMemoire (Hervé, 2022).

Prior to analysis, a semi-landmark repeatability test was conducted to ensure that landmarks were placed consistently across all images and models. Landmarks were placed on a

randomly selected set of five mandibles and five crania, one set placed on each specimen over the course of five days. A generalized Procrustes superimposition (GPA) was performed followed by principal component analysis (PCA) on the Procrustes coordinates. Procrustes ANOVAs were performed to assess differences between the landmark sets.

An analysis was performed for the entire landmark configuration of the crania but also separately for each curve of the crania (dorsal midline, orbit, temporalis scar and zygomatic arch). The whole crania sample allows exploration of morphological variation across the entire skull between populations, sex and year collected. Separate analysis of each curve allowed inclusion of specimens that were missing or damaged and could not be included in the whole crania sample. In total, 113 specimens were analysed in the whole crania subset. For the curves of dorsal midline, orbit, zygomatic arch, and temporalis scar, 161, 150, 143, 154 specimens were included respectively. 388 mandibles were analysed separately from the crania, with any broken specimens or specimens with missing landmarks being excluded entirely.

Prior to registration, centroid sizes (the square root of the sum of squared distances of all points of the landmark configuration to their centroid) were calculated for each specimen for use in size comparisons. A generalized Procrustes superimposition (GPA) was performed to standardize the size, position, and rotation of landmark configurations. This was then followed by principal component analysis (PCA) on the Procrustes coordinates, which summarises shape variation along the main axes of the morphospace. It was expected that if there was significant anatomical variation between populations and year groups that these subsets would occupy different regions of the morphospace, with some overlap of populations with similar genetic backgrounds. Procrustes ANOVAs were performed to assess differences between groups, focusing on differences in shape and size between populations, decade collected and sex. Shape changes along each principal component axis were visualised in MorphoJ. Non-parametric ANOVAs were employed to assess the effect of each variable on individual principal components. The relationship between shape and size was assessed through ANOVA, and on each principal component using the `lm.rpp` function in R package RRPP (version 0.2.0). It was then assessed whether the allometric trajectories of each population followed a common allometric trajectory (model; $\text{shape} \sim \text{size} + \text{population}$) or followed unique allometric trajectories ($\text{shape} \sim \text{size} * \text{population}$). Differences between these models were compared through ANOVA. A significant difference tells us that unique

allometric trajectories is the correct model. Once established, post hoc pairwise permutation tests were performed to establish the differences in trajectories between populations.

2.2.3 Mechanical Advantage

The functional significance of morphological variance between the populations was estimated using mechanical advantage (hereafter MA) of the temporalis muscle, one of the main muscles of mastication. Previous work (Cox et al. 2020) analysed the three main major masticatory muscles – the temporalis, superficial masseter and deep masseter and found that there was a significant difference in the MA of the dorsally inserting temporalis fibres of Formby red squirrels and all other groups. Additionally, the MA of the ventrally inserting temporalis fibres was found to be different between the Scottish and Northern English populations and all other populations. No differences in MA of the superficial or deep masseters between populations were detected. As the temporalis muscle was the only muscle to show differences in MA between populations, the temporalis muscle MA only was re-analysed in this study. In Cox et al. 2020, the lateral side of the mandible was landmarked, whereas, in this study, the medial side was landmarked, making it more difficult to accurately take the linear measurements needed to calculate the MA of the masseter muscles.

MA was estimated from the linear measurements of the jaw (as described in Casanovas-Vilar & van Dam, 2013; Cox et al., 2020), calculated as the ratio of the muscle in-lever to the incisor biting out-lever. Two in-lever measurements were taken for the temporalis muscles - from the most dorsally inserting fibres at the tip of the coronoid process to the tip of the condylar process (T_d) and from the most ventrally inserting fibres at the base of the coronoid process to the tip of the condylar process (T_v). The biting out-lever was recorded as the distance between the tip of the condylar process and the point at which the incisor erupts from the mandible (see Figure. 2.4). The impact of population on temporalis muscles MA was assessed using ANOVA undertaken in R. Although helpful in estimating bite force, there are a number of caveats to this method. Firstly, MA is being calculated using lever-arms (as opposed to using moment arms which is the more correct method when calculating MA). Moment arms can be measured through articulation of the mandible with the skull. However, including only specimens that had both an intact cranium and mandible would have significantly reduced the dataset. Additionally, it was preferable to continue using methods as described in Cox et al. (2020) to allow for more reliable comparison. Previous research has chosen to use lever-arms over moment arms as an approximation of MA (Casanovas-Vilar &

van Dam, 2013; Renaud et al., 2015; Gomes Rodrigues et al., 2016; Jones et al., 2018; West & King, 2018) and this work follows these methods closely. A second caveat is that muscles usually have large attachment sites, rather than a single point as used here. But this over-simplification is deemed necessary to be within the scope of this project. Lastly, no lateral variation in landmarks could be accounted for as measurements were made from 2D images. However, red squirrel hemi-mandibles are virtually planar due to their sciurognathous condition (Hautier et al., 2011). As such, despite the over-simplification of this method, the results can still be considered meaningful.

2.3 Results

2.3.1 – Cranial Size Variation

Results of the crania landmarking repeatability tests showed landmarks sets for the same specimen were significantly different to other specimens landmark sets, and were not significantly different to landmark sets of the same specimen in shape (Procrustes ANOVA: $F = 0.39$, $p = 1.2$). Landmark sets of the same specimen were grouped together on a principal components plot.

Analysis showed significant difference in cranial centroid size between the three populations of North Scotland, Borders and Formby (c. Specimens from Formby have the largest crania, and the Northern English and South Scottish specimens are the smallest (see Figure 2.5). No difference in cranial centroid size was detected between sexes.

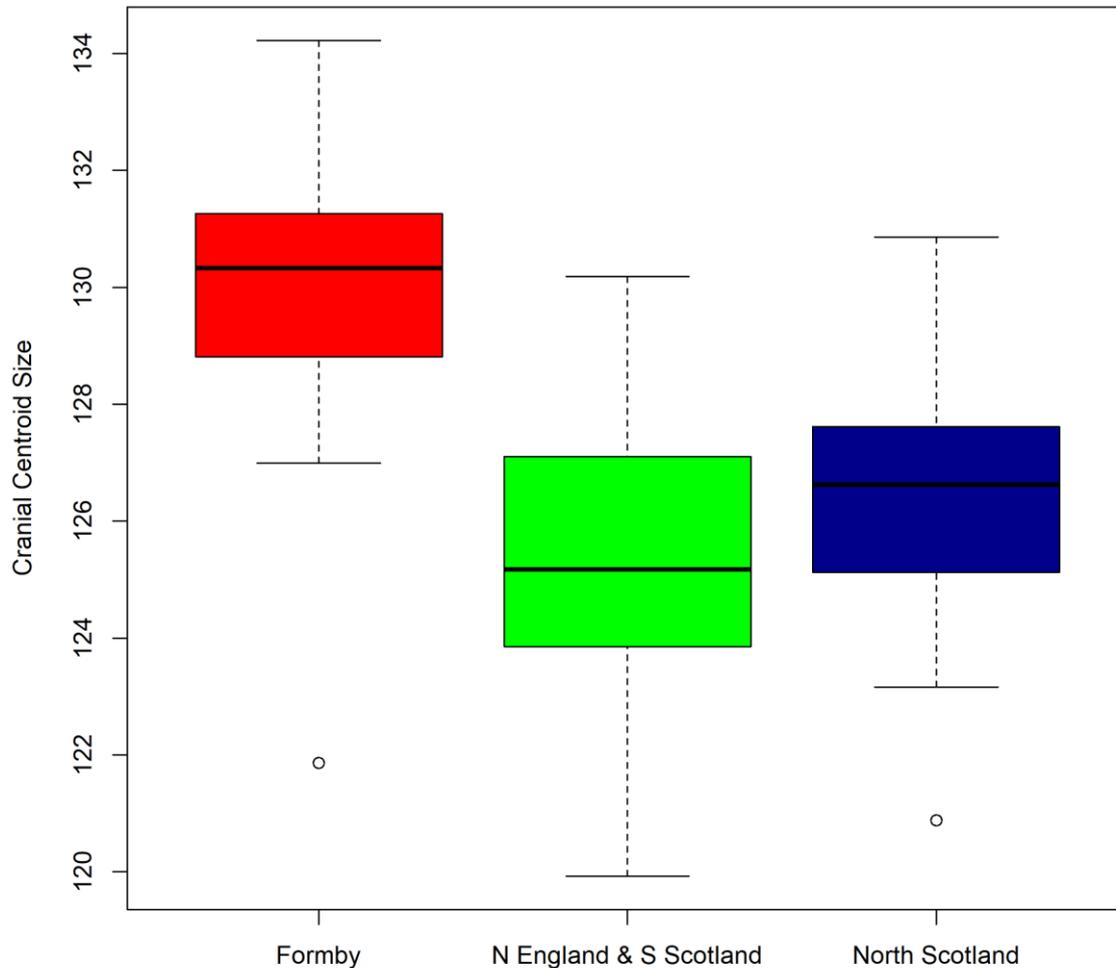


Figure 2.5 Boxplot of Whole Crania centroid size in British red squirrel populations. Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box. Populations are Formby (n= 61), Northern England and Southern Scotland (n= 25) and Northern Scotland (n= 27).

2.3.2 – Cranial Shape Variation

Principal components analysis of the sample of 112 fully intact cranial specimens showed the first three PCs represented 37.6% of variation (PC1 – 15.6%, PC2 – 14.6% and PC3 – 7.4%). Separation by population was seen clearly along PC1 and PC2 (see Figure 2.6). There is significant overlap of populations, which is expected in intraspecific variation. Shape variation between populations is confirmed through Procrustes ANOVA on all coordinates ($F = 8.18, p = 0.001$) with detectable shape variation along PC1 ($F = 12.69, p = 0.0001$) and PC2

($F = 78.84$, $p > 0.001$). Procrustes ANOVA shows a significant relationship of cranial size on shape ($F = 9.98$, $p = 0.001$). Further analysis shows that this is evident on PC2 only ($F = 110.36$, $p < 0.001$). ANOVA between the common allometric trajectory (shape \sim size * population) and unique allometric trajectories (shape \sim size + population) showed no significant difference ($F = 1.3$, $p = 0.08$), establishing there is no significant difference in allometric trajectories between populations.

Interestingly, sexual dimorphism was detected through Procrustes ANOVA ($F = 1.72$, $p = 0.044$). This shape difference is detected along PC3 ($F = 6.38$, $p = 0.0135$). Further analysis by Procrustes ANOVA shows that differences in shape between sexes is supported only within specimens from Formby ($F = 1.99$, $p = 0.005$).

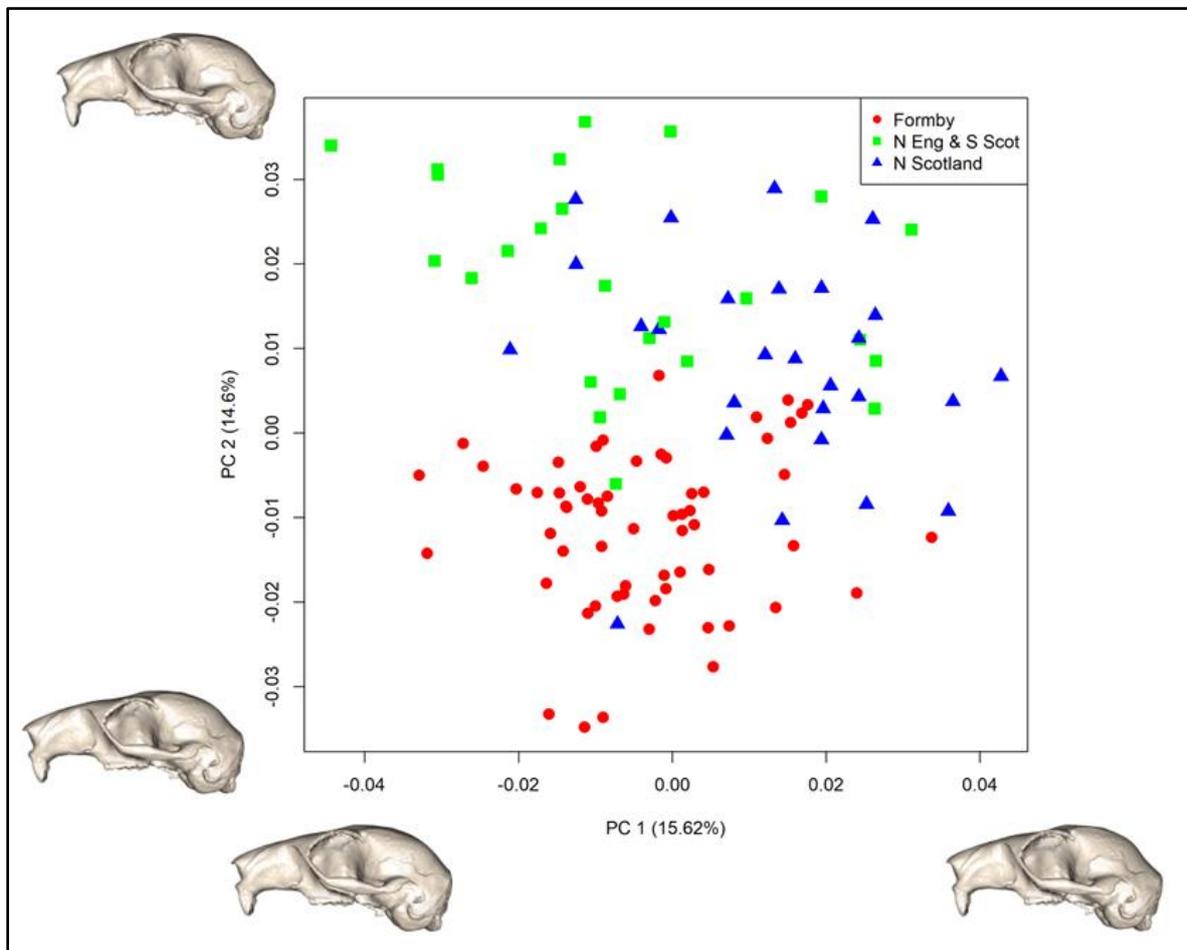


Figure 2.6. Plot of PC1 vs PC2 from principal components analysis of whole cranial shape of British red squirrels. Each data point represents the shape data associated with a single specimen. Data points are coloured and shaped by population. This dataset represents specimens with intact crania where all 72 landmarks could be placed: 112 specimens in total.

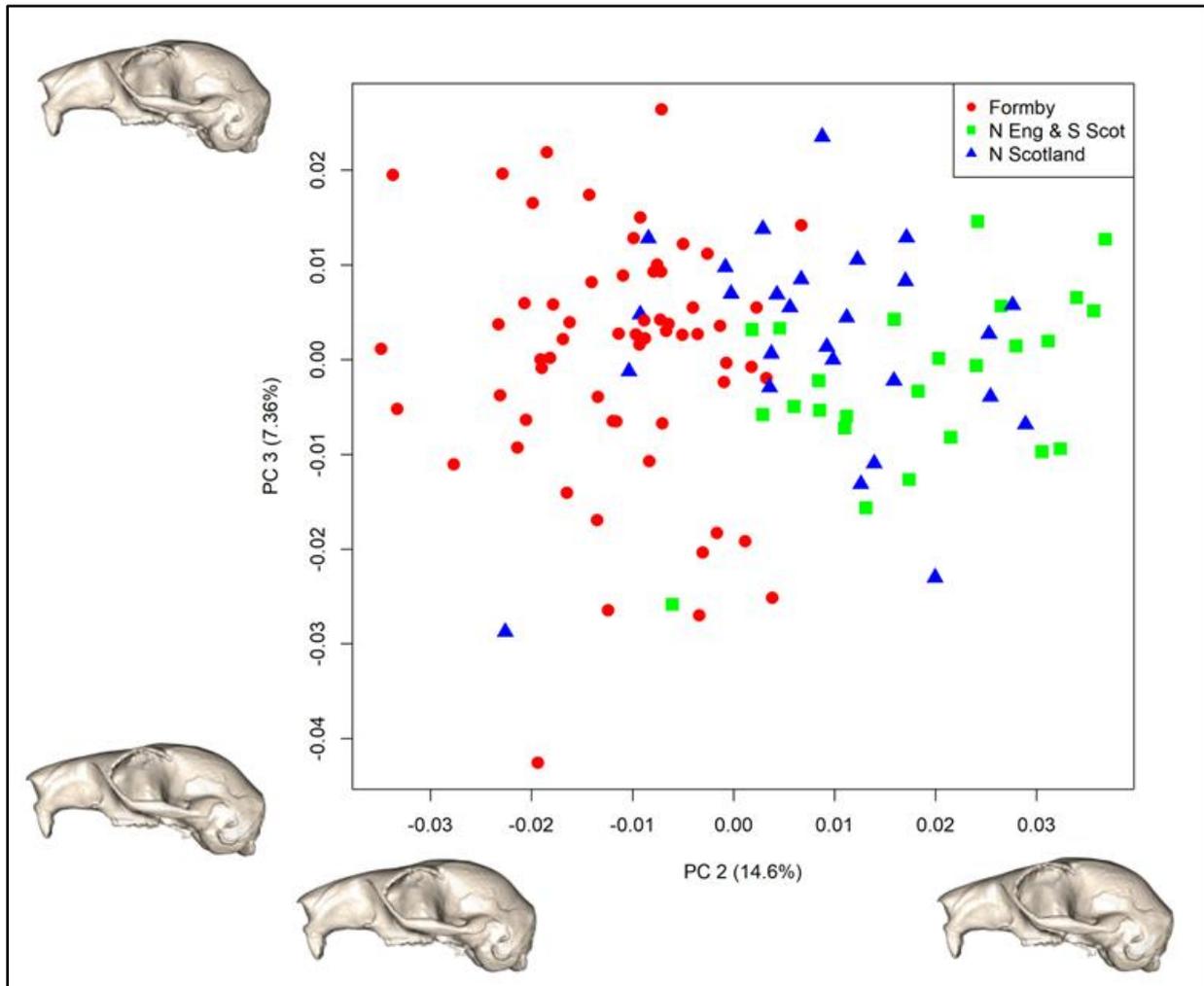


Figure 2.7. Plot of PC2 vs PC3 from principal components analysis of whole cranial shape of British red squirrels. Each data point represents the shape data associated with a single specimen. Data points are coloured and shaped by population. This dataset represents specimens with intact crania where all 72 landmarks could be placed: 112 specimens in total.

PC1, from negative to positive values (see Figure 2.7), shows a trend from a high and rounded to a more flattened cranial vault, with the orbit becoming increasingly large relative to the rest of the skull. North Scottish specimens are most densely concentrated in the positive values of PC1, suggesting that North Scottish specimens are more likely to have a higher cranial vault compared to Formby and Borders specimens. The temporalis scar is positioned ventrally in the negative values of PC1 and more dorsally with a steeper angle before converging with the dorsal-most point of the dorsal midline (Landmark 3). It would therefore be expected that North Scottish specimens will generally have a more dorsally positioned temporalis scar. This creates more surface area for the attachment of the

temporalis muscle, and potential for a larger masseter muscle. PC2 from negative to positive also sees a trend from a flatter to taller cranial vault, an increasingly large orbit relative to the rest of the skull and a more dorsally positioned temporalis scar. This overall distribution shows that Formby specimens, occupying the negative end of the axes of PC1 and PC2, have a uniquely flattened cranial vault, a ventrally positioned temporalis scar and a smaller orbit (relative to cranial size) than either Northern English/South Scottish and North Scottish specimens.

Zygomatic arch variation ranges in morphology along PC2. In the negative values the anterior portion of the zygomatic arch is more laterally flared (in the zygomatic curve landmarks 1 – 6) compared to the positive values. There is also a slight lateral flaring in the posterior zygomatic arch in the negative values of PC2, which results in a steep angled junction of the zygomatic arch before re-joining with the glenoid fossa. Separation of Formby specimens from other populations along PC2 indicates that this more angular zygomatic arch is another unique shape trait of Formby specimens.

2.3.3 – Cranial Shape Variation – Isolated Curves

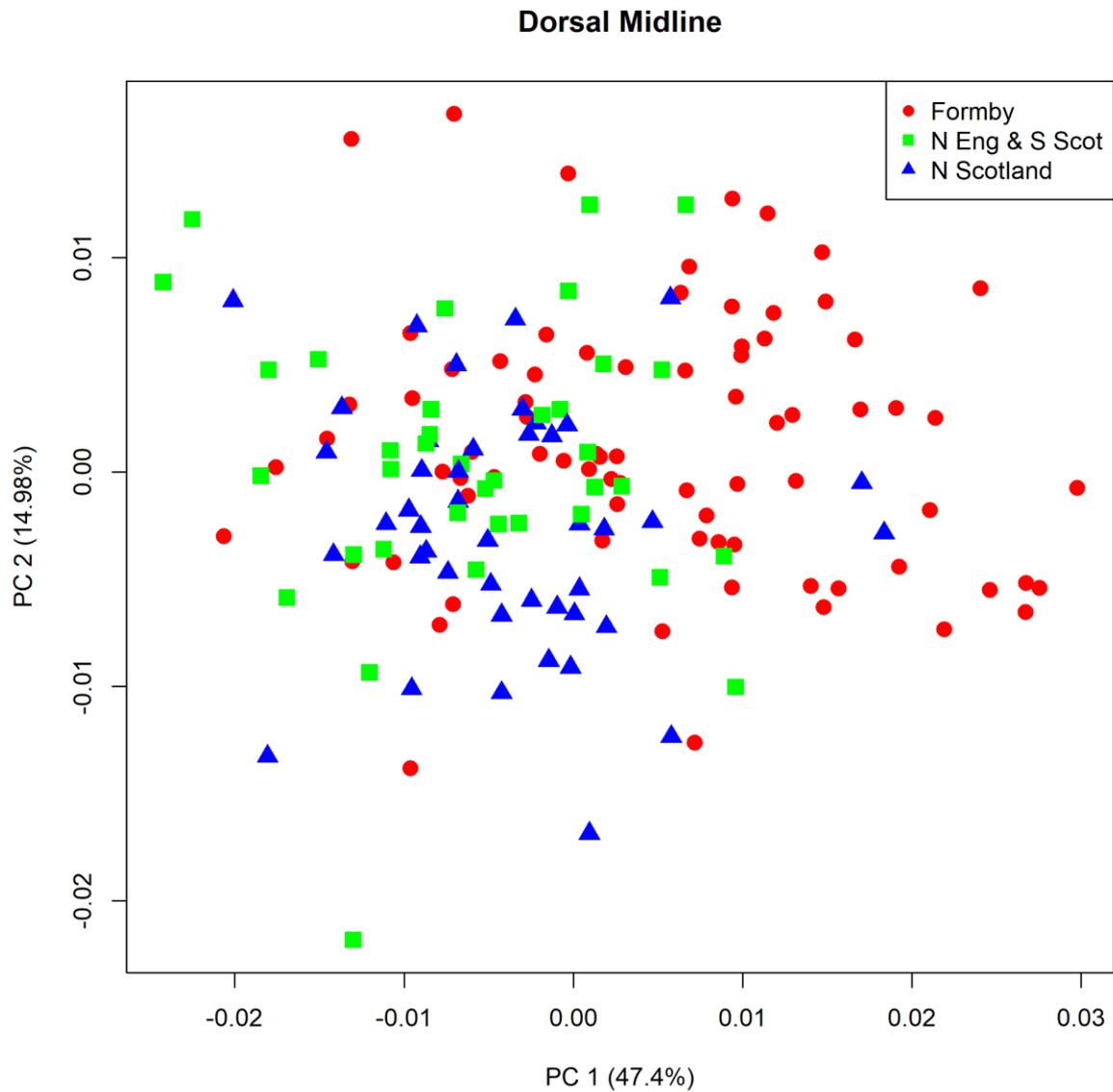


Figure 2.8. Plot of PC1 vs PC2 from principal components analysis of dorsal midline shape of British red squirrels. Each data point represents the shape data associated with a single specimen, based on 14 equidistantly placed semilandmarks. $n = 160$. Data points are coloured and shaped by population.

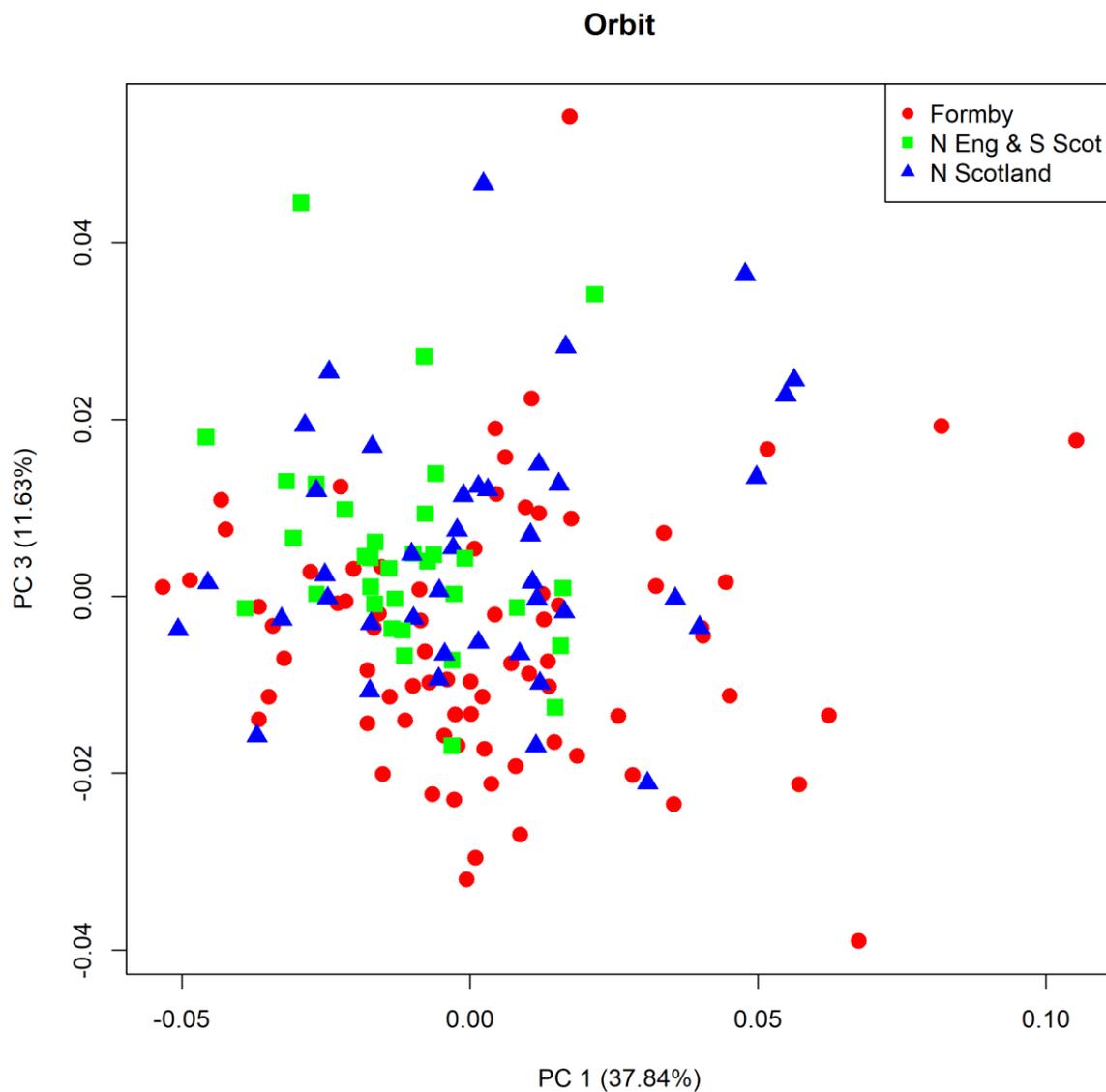


Figure 2.9. Plot of PC1 vs PC3 from principal components analysis of orbit shape of British red squirrels. Each data point represents the shape data associated with a single specimen, based on 10 equidistantly placed semilandmarks. n = 150. Data points are coloured and shaped by population.

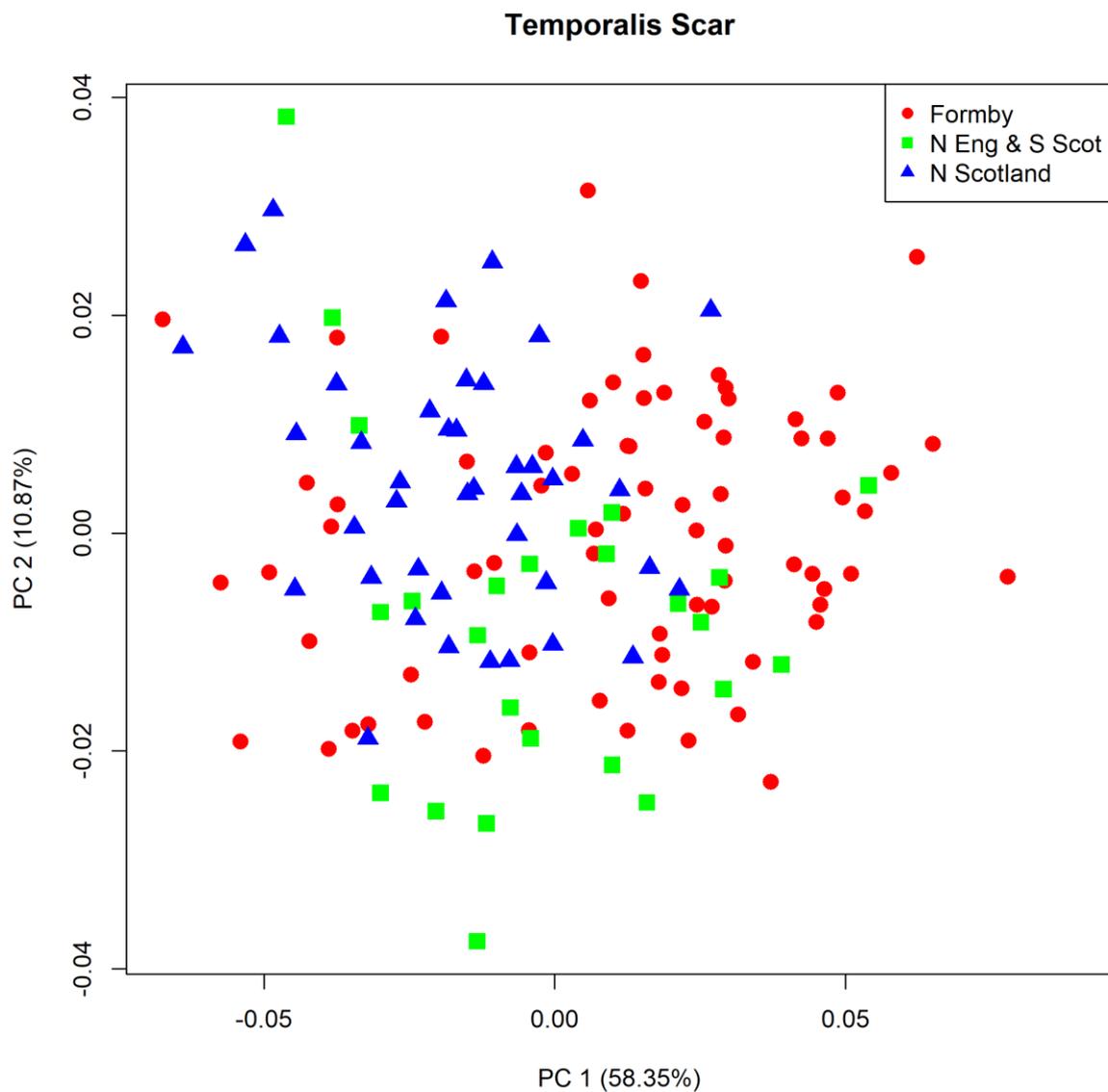


Figure 2.10. Plot of PC1 vs PC2 from principal components analysis of shape of the cranial temporalis scar of British red squirrels. Each data point represents the shape data associated with a single specimen, based on 15 equidistantly placed semilandmarks. n = 155. Data points are coloured and shaped by population.

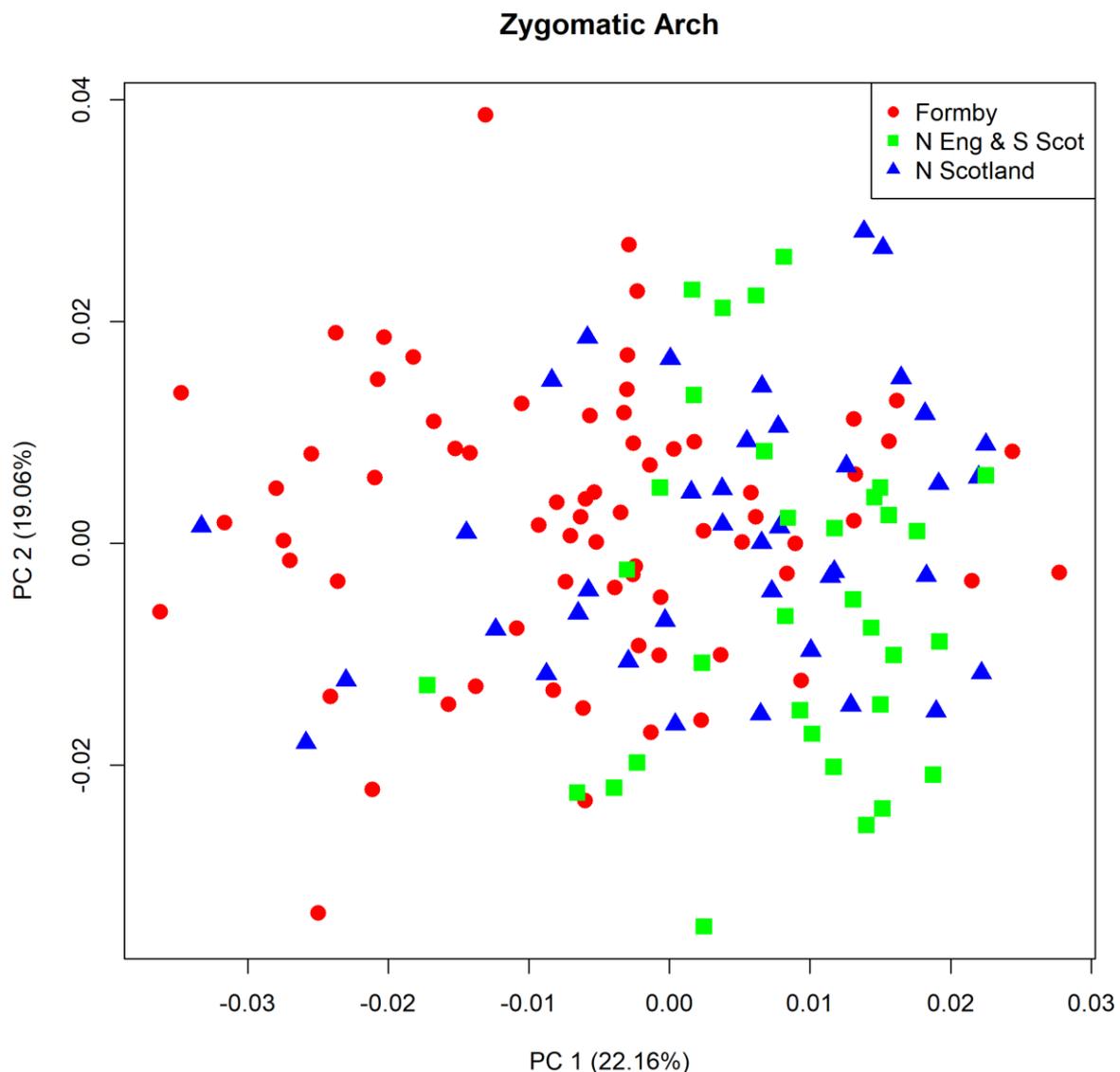


Figure 2.11. Plot of PC1 vs PC2 from principal components analysis of zygomatic arch shape of British red squirrels. Each data point represents the shape data associated with a single specimen, based on 18 equidistantly placed semilandmarks. n = 144. Data points are coloured and shaped by population.

Further analysis of portions of the crania detected significant shape variation by populations across all four curves: dorsal midline ($F = 9.95$, $p = 0.001$), orbit ($F = 4.68$, $p = 0.001$), temporalis scar ($F = 8.17$, $p = 0.001$) and zygomatic arch ($F = 6.58$, $p = 0.001$) shown in PC plots in Figures 2.8, 2.9, 2.10, 2.11. No sexual dimorphism was detected in the dorsal midline ($F = 0.69$, $p = 0.689$), orbit ($F = 1.72$, $p = 0.084$) or temporalis scar ($F = 1.32$, $p = 0.221$). However, a difference in shape between sexes was detected on the zygomatic arch ($F = 2.56$,

$p = 0.013$). This is seen along PC4 ($F = 5.74$, $p = 0.0165$), PC4 accounts for 10.9% of overall variation within the sample. Further investigation shows that this difference in shape is found within specimens from Formby only ($F = 2.14$, $p = 0.039$). The shape of all four curves is unrelated to the overall cranial centroid size, as detected through Procrustes ANOVA ($p > 0.07$ for all four curves).

Shape variation in the dorsal midline, sees a change in the cranial vault from high/pronounced to flattened along PC1, with Formby specimens occupying the flatter end of the distribution and Northern England and Scotland occupying the higher cranial vault end of the PC plot. The orbit ranges along PC1 from a flatter shape to more rounded. PC3 shows limited shape variation and the separation of the populations (Formby occupying the negative values, and North Scotland and Borders are occupying the positive values) is more likely to be related to overall orbit size (as confirmed with Procrustes ANOVA $F = 7.05$, $p = 0.0086$). The temporalis scar shows a shape change along PC1 from a steep ventral curve towards the base of the cranium in the negative values (where mostly Scottish and Northern English specimens plot) to a less steep curve in the positive values (where Formby specimens mostly plot). Along PC3 there is similar shape change from a relatively straight temporalis scar in the negative values (where mostly Northern English/South Scottish specimens plot) and becomes increasingly convex in shape towards the positive values (where mostly North Scottish specimens plot). This results in an overall distribution where Northern Scottish specimens have a more dorsally positioned temporalis scar and Formby specimens have a more ventrally positioned temporalis scar, suggesting a morphology where the temporalis muscle covers more surface area of the cranium in North Scottish specimens relative the Formby specimens. The zygomatic arch has a more curved shape in the portion of the PC plot mainly populated by Formby specimens, and along PC1 becomes more flattened. The zygomatic arch changes in shape along PC1 where the junctions of the jugal and the maxillary (first and second semi-landmarks) and the squamosal and rest of cranium (15th to 18th semilandmarks) are more angular in the negative values (where mostly Formby specimens plot) to a smoother overall curve in the positive values (where the other populations plot).

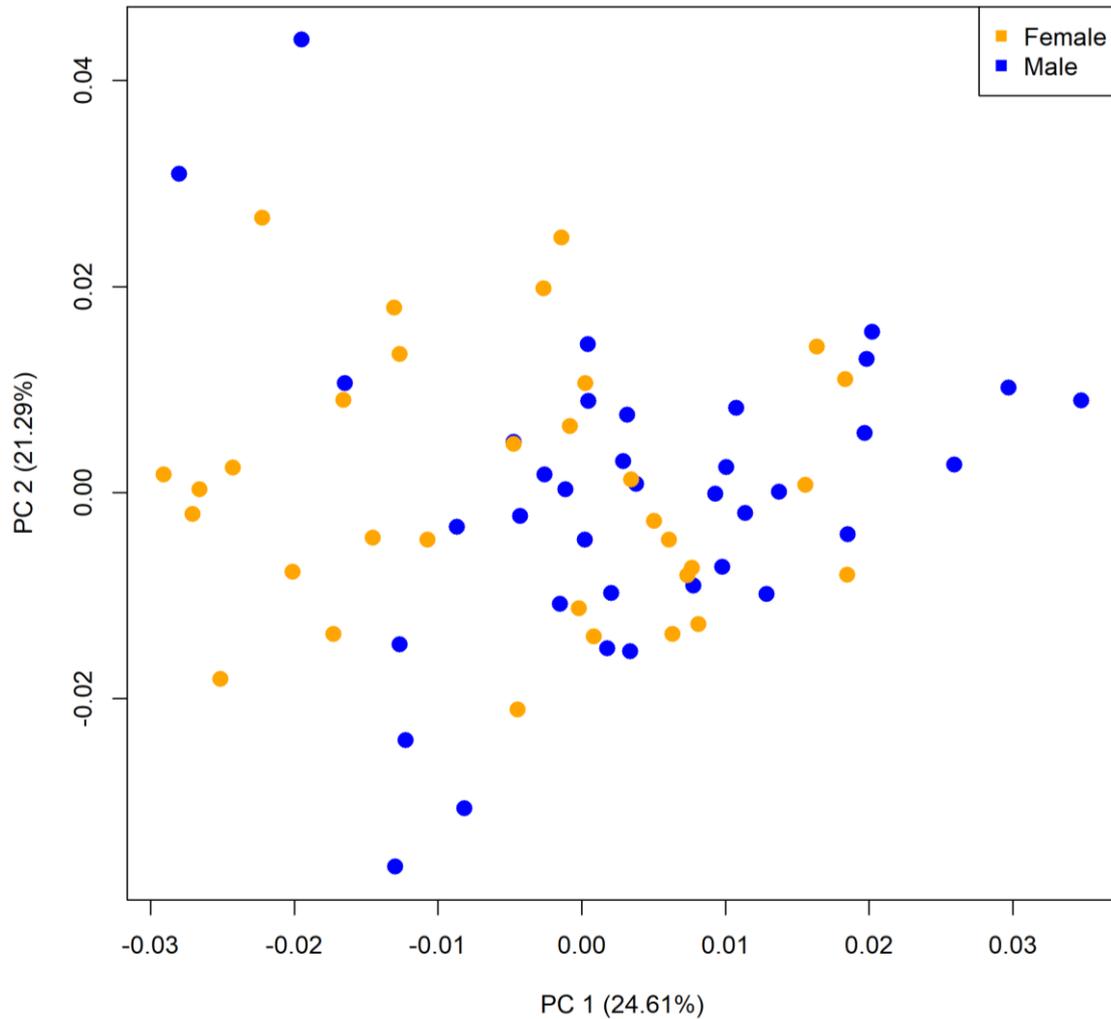


Figure 2.12. Principal components plot of the zygomatic arch of Formby specimens only. Each data point represents shape data of the zygomatic arch for an individual specimen. Data coloured by sex of specimens. N= 70 (32 female, 38 male).

Principal component analysis of the zygomatic arch of Formby specimens shows considerable overlap of male and female specimens (see Figure 2.12), however ANOVA did detect a difference in morphology between sexes along PC1 ($F = 7.35$, $p = 0.0091$). This shows similarities to previous differences in of a change in shape from a smoother overall curve of the zygomatic arch to more angular junctions of the jugal with the maxillary and the squamosal with the rest of cranium, from negative to positive. In this case male specimens are more likely to have the smoother curve (plotting in the negative values of PC1) and females have the more angular curve (plotting in the positive values).

2.3.4 – Cranial Size and Shape Variation Over time

No significant size difference was detected over time within North Scottish Specimens ($F = 0.23$, $p = 0.65$). However, variation in shape was detected between Formby specimens collected in 1990s and those collected in 2010s (Procrustes ANOVA $F = 4.06$, $p = 0.001$) (see Figure 2.13). Cranial shape variation between sexes was supported by Procrustes ANOVA in specimens collected in the 2010s ($F = 1.8$, $p = 0.02$) but not in specimens collected in 1990s ($F = 1.37$, $p = 0.14$).

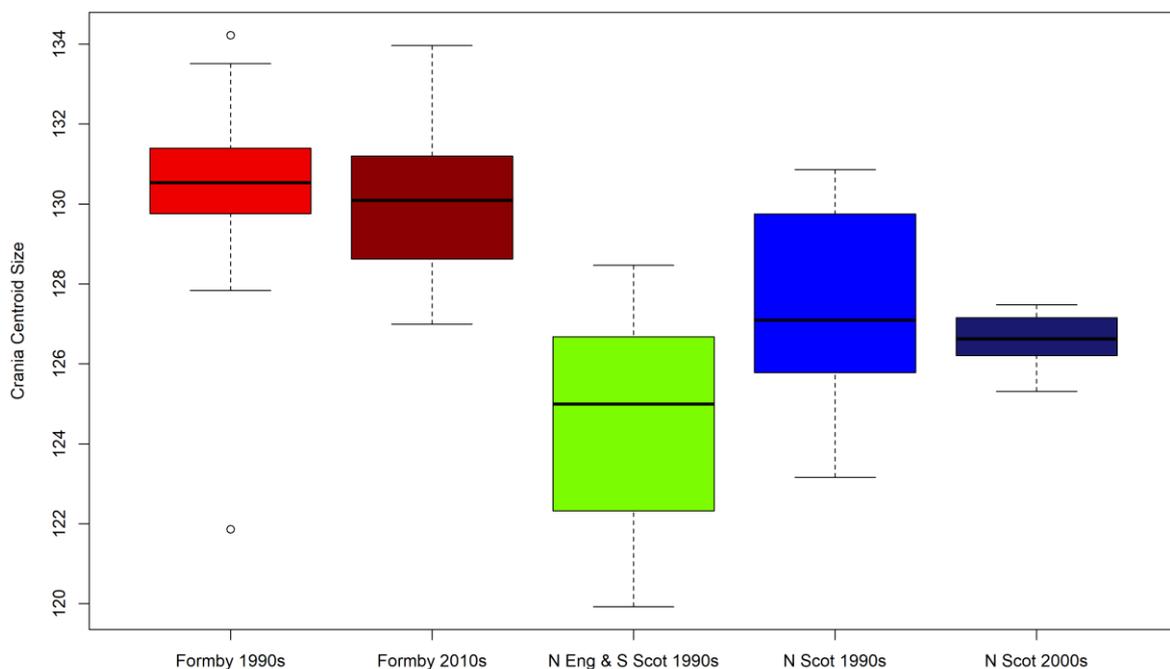


Figure 2.13 Boxplot of crania log centroid size in British red squirrel populations over different decades. Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box. Populations included are Formby 1990s ($n = 13$), Formby 2010s ($n = 48$), Borders 1990s ($n = 18$), Northern Scotland 1990s ($n = 14$) and Northern Scotland 2000s ($n = 6$).

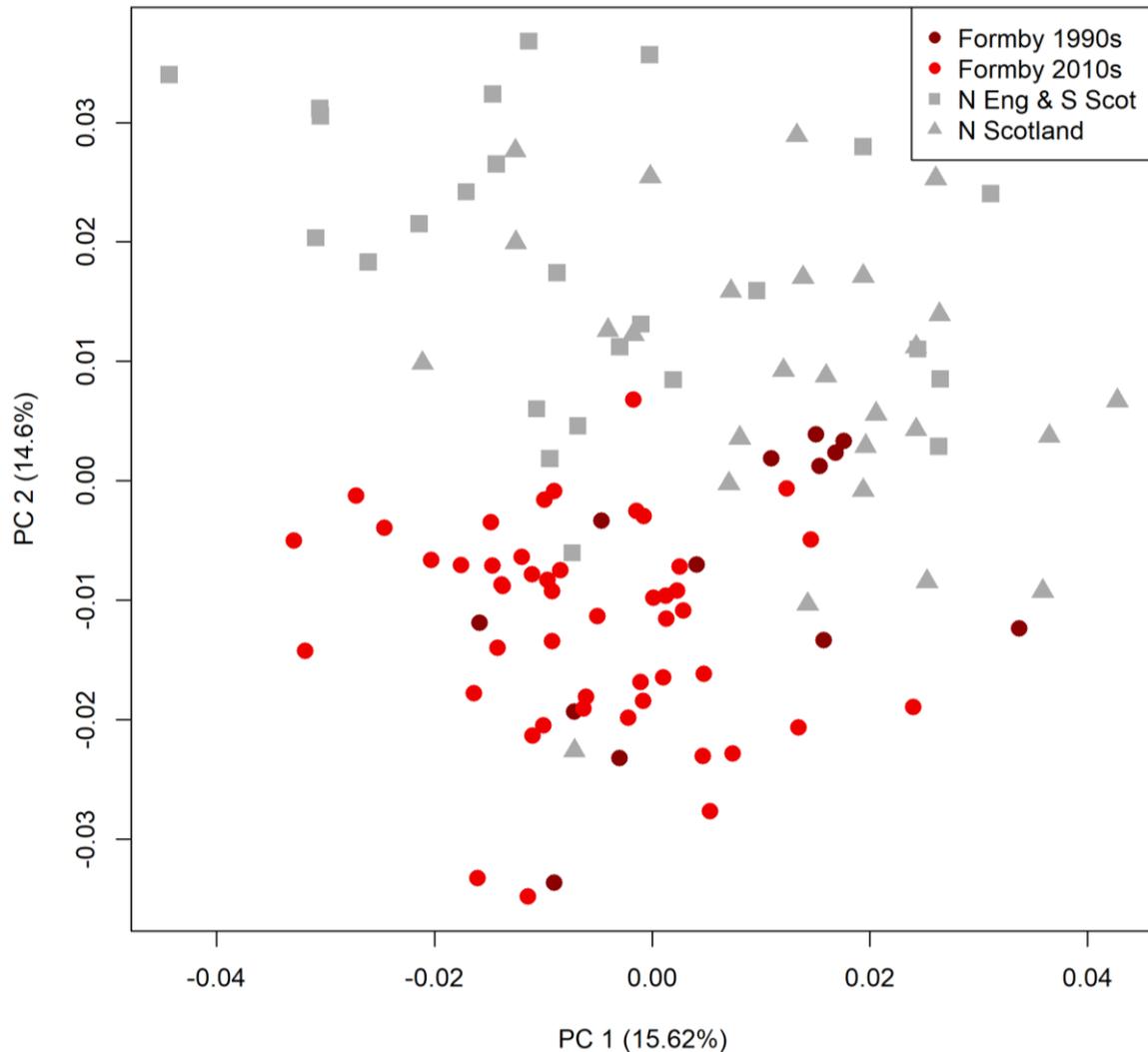


Figure 2.14. Plot of results of principal components analysis of whole crania. Specimens other than those from Formby are coloured in grey. Formby specimens are coloured by decade.

Previous detection of sexual dimorphism in the zygomatic arch of Formby specimens prompted a comparison of between specimens from Formby collected in the 1990s and 2010s. This showed that the sexual dimorphism in the zygomatic arch was not detected in specimens from 1990s ($F = 0.69$, $p = 0.699$) but was in specimens from 2010s ($F = 2.46$, $p = 0.013$). This is mimicked in analysis of the crania as a whole which shows that Formby specimens from 1990s do not show significant differences in morphology between sexes 1990s ($F = 1.06$, $p = 0.14$), but specimens from 2010 do ($F = 1.8$, $p = 0.02$).

2.3.5 - Mandibular Size Variation

Results of the mandible landmarking repeatability tests showed landmarks sets for the same specimen were significantly different to other specimens landmark sets, and were not significantly different to landmark sets of the same specimen in both size (Procrustes ANOVA: $F = 1.90$, $p = 0.18$) and shape ($F = 0.49$, $p = 1.0$). Landmark sets of the same specimen were grouped together on a principal components plot.

Mandible centroid size shows significant variation between populations ($F = 3.88$, $p = 0.014$). Mean centroid sizes of each population show Jersey mandible specimens to be largest followed by mandible specimens from Formby (see Figure 2.15). Specimens from the Borders were the smallest. No size difference between sexes was detected when the whole sample was analysed (Procrustes ANOVA, $F = 1.64$, $p = 0.205$).

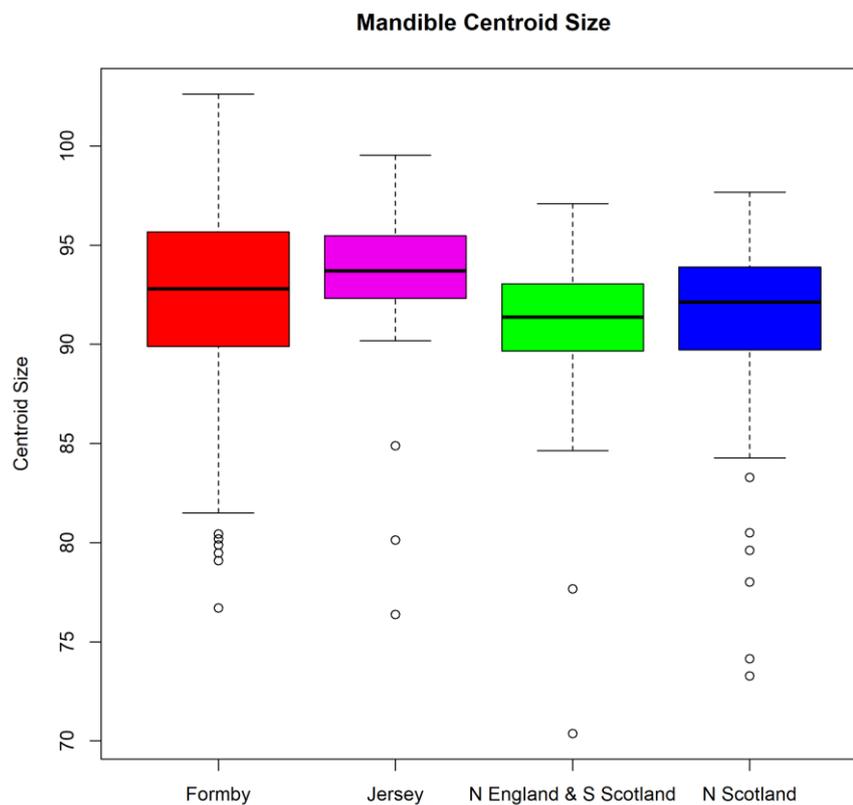


Figure 2.15. Boxplot of mandible log centroid size in British red squirrel populations over different decades. Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range. Populations are Formby (n= 166), Jersey (n= 37), Borders (n= 93) and North Scotland (n= 77).

Mandible Size of Populations by Decade

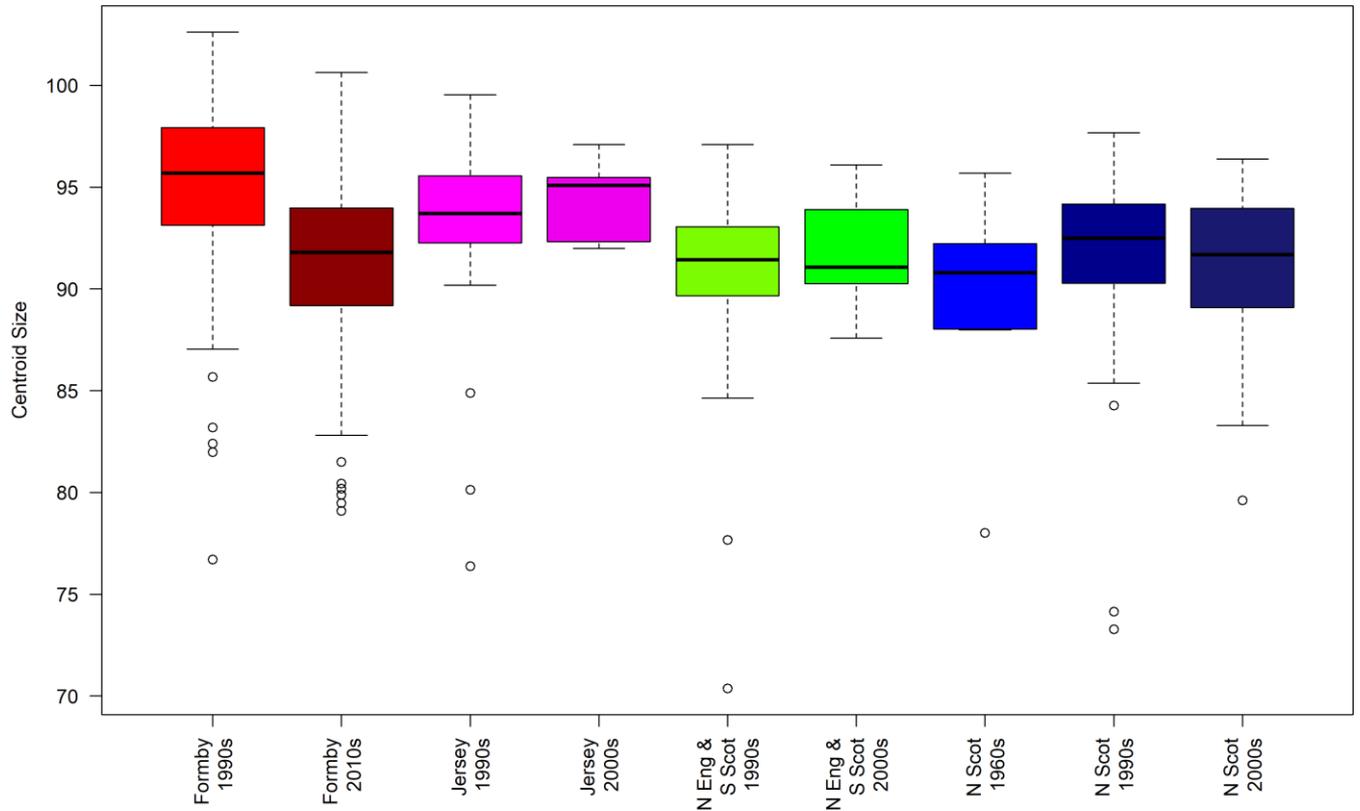


Figure 2.16. Boxplot of mandible log centroid size in British red squirrel populations over different decades. Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range. Populations are Formby 1990s (n= 61), Formby 2010s (n= 105), Borders 1990s (n= 78), Borders 2000s (n= 12), Jersey 1990s (n= 27), Jersey 2000s (n= 10), North Scotland 1960s (n= 11), North Scotland 1990s (n= 58) and North Scotland 2000s (n= 15).

2.3.6 – Mandibular Shape Variation

Principal components analysis of a sample of 391 mandible specimens showed the first three PCs represent 64.3% of variation (PC1 – 34.1%, PC2 – 22.2% and PC3 - 7.9%). Separation by population was seen clearly along PC1 ($F = 4.80$, $p = 0.0023$), PC2 ($F = 167.07$, $p < 0.001$) and PC3 ($F = 7.65$, $p < 0.001$) (see Figure 2.17). There is significant overlap of populations, which is expected in intraspecific variation, however least overlap is seen in North Scotland and Formby. Shape variation between populations is confirmed through Procrustes ANOVA on shape coordinates ($F = 24.61$, $p = 0.001$). No sexual dimorphism was detected for shape ($F = 1.36$, $p = 0.166$).

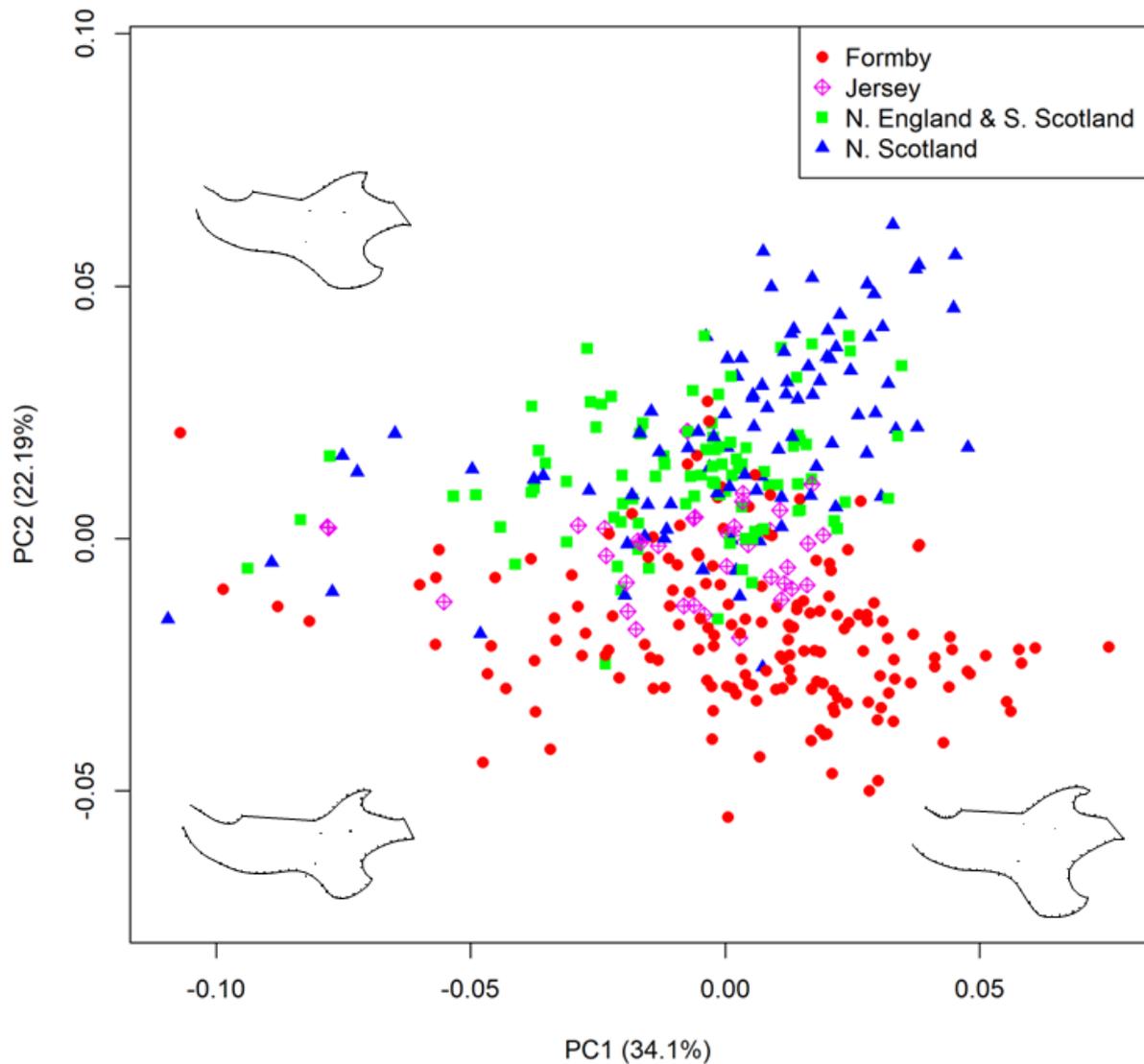


Figure 2.17. Plot of results of principal components analysis of mandible, showing principal components 1 and 2. Each data point represents the shape data associated with a single specimen. Data points are coloured and shaped by population.

PC1 represents a general lengthening of the mandible from negative to positive values, with the lengthening most evident in the diastema. There is also a loss in depth of the mandible with this lengthening. Along PC2 the coronoid process ranges in shape from highly curved (negative values) to short, blunt, and less well defined (positive values). The inflection between the condylar and angular processes ranges from deep (negative values) to more shallow

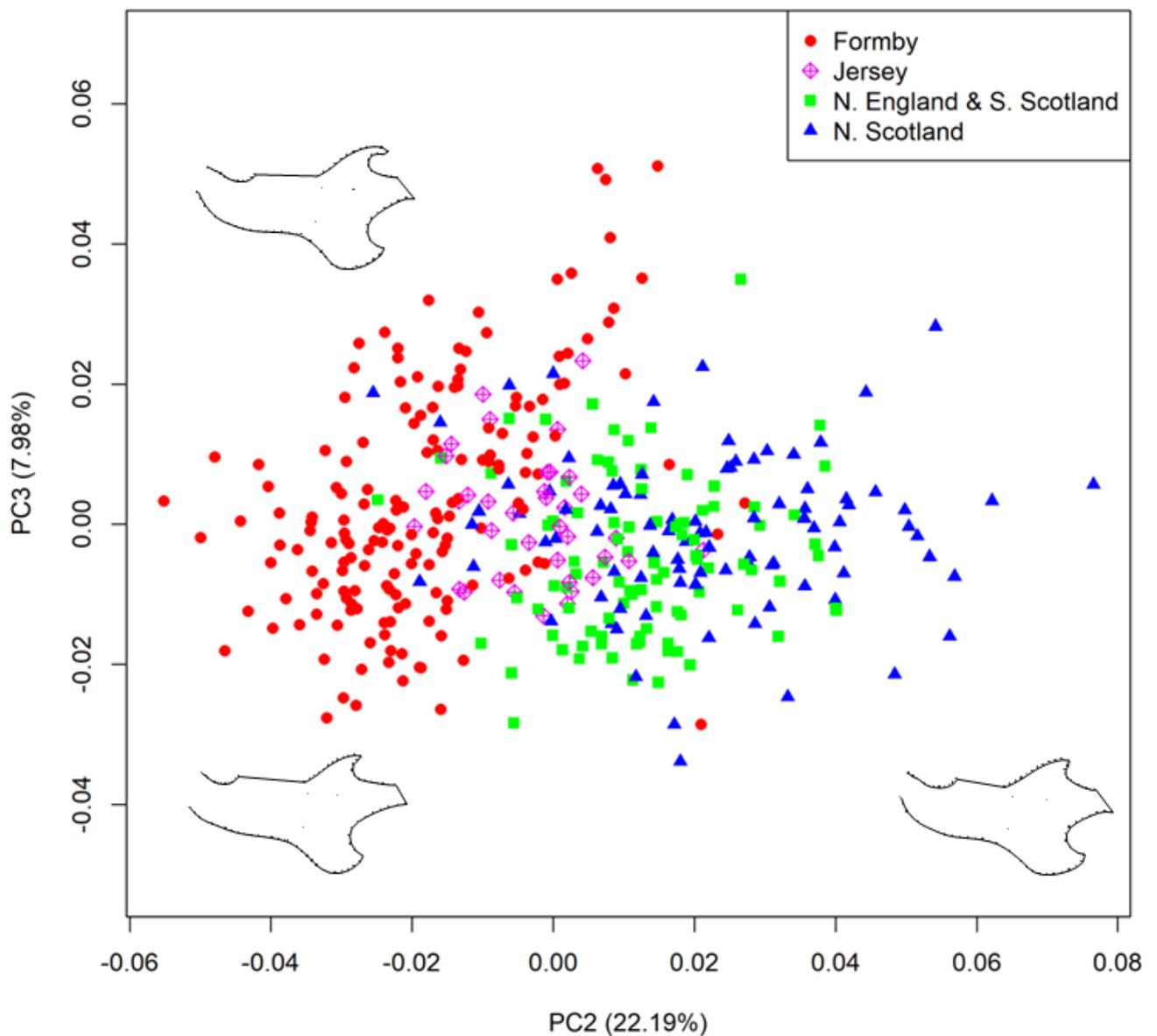


Figure 2.18. Plot of results of principal components analysis of mandible, showing principal components 2 and 3. Each data point represents the shape data associated with a single specimen. Data points are coloured and shaped by population.

PC3 represents a change from a blunt to curved coronoid process from the negative to positive values, and, similar to PC1, an overall narrowing and lengthening of the mandible, particularly in the diastema. Most specimens occupy the area between -0.02 and 0.02 along PC3, and therefore show an intermediate morphology (see Figure 2.18).

North Scottish specimens mostly occupy the negative values of the axis and therefore are quite deep, have a less defined coronoid process and a deep inflection between the condylar

and angular processes, whereas Formby specimens, on the positive end of the axis, are generally narrower with a highly curved coronoid process and shallow inflection between the condylar and angular processes. Specimens from the Borders have a similar morphology to Northern Scottish squirrels, so are generally deep, short and have blunt coronoid processes. Specimens from Jersey are located more centrally in the graph and are therefore more likely to show an intermediate morphology.

2.3.7 – Mandibular Allometry

Procrustes ANOVA shows a significant relationship of mandible size on shape ($F = 59.537$, $p = 0.001$). Further analysis shows that this is evident on PC1 ($F = 180.22$, $p < 0.001$) and PC3 ($F = 41.91$, $p < 0.001$), but not PC2. Unique allometric trajectories for the different populations were established and therefore the model: $\text{shape} \sim \text{size} * \text{population}$ could be used for further post hoc permutation tests. These tests showed a significant difference in slope between trajectories of North Scottish specimens and all other populations ($p = 0.001$, $p = 0.01$, $p = 0.038$ for Formby, Jersey and Borders respectively). Figure 2.19 shows that with increasing size, North Scottish specimens increase depth of the mandible.

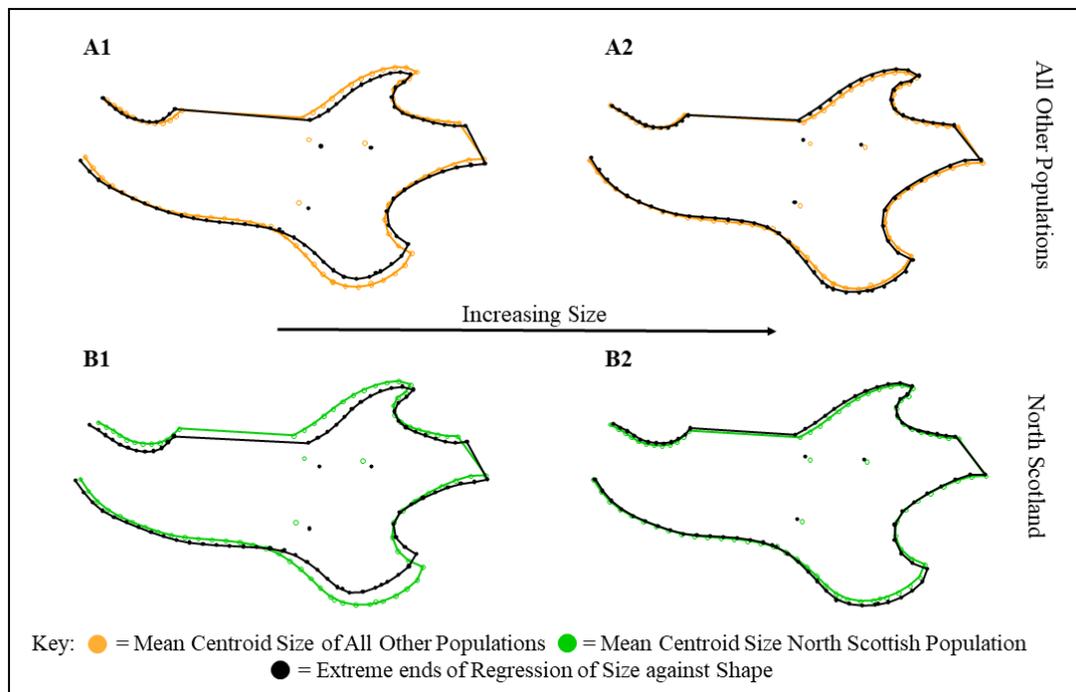


Figure 2.19. Wireframes of mandibular landmarks showing the difference of allometric trajectories of Formby, Jersey and Northern English/South Scottish specimens (A1 and A2) and the North Scottish specimens (B1 and B2). Images represent the morphology of specimens of the extreme ends of the regression analysis.

2.3.8 – Mandibular Shape Variation Over Time

Shape variation was detected between year groups within the Formby population only ($F = 34.53$, $p = 0.001$). A difference in size between specimens collected in 1990s and 2010s was also detected in the Formby sample ($F = 23.47$, $p = 0.001$) with specimens from 1990s being significantly larger. It was not possible to analyse change over time for the whole sample as some decades are only represented by a single population. As previously shown, population has a significant effect on the morphology of a specimen. As such, populations were analysed separately and only decades that were represented by more than 10 specimens were included.

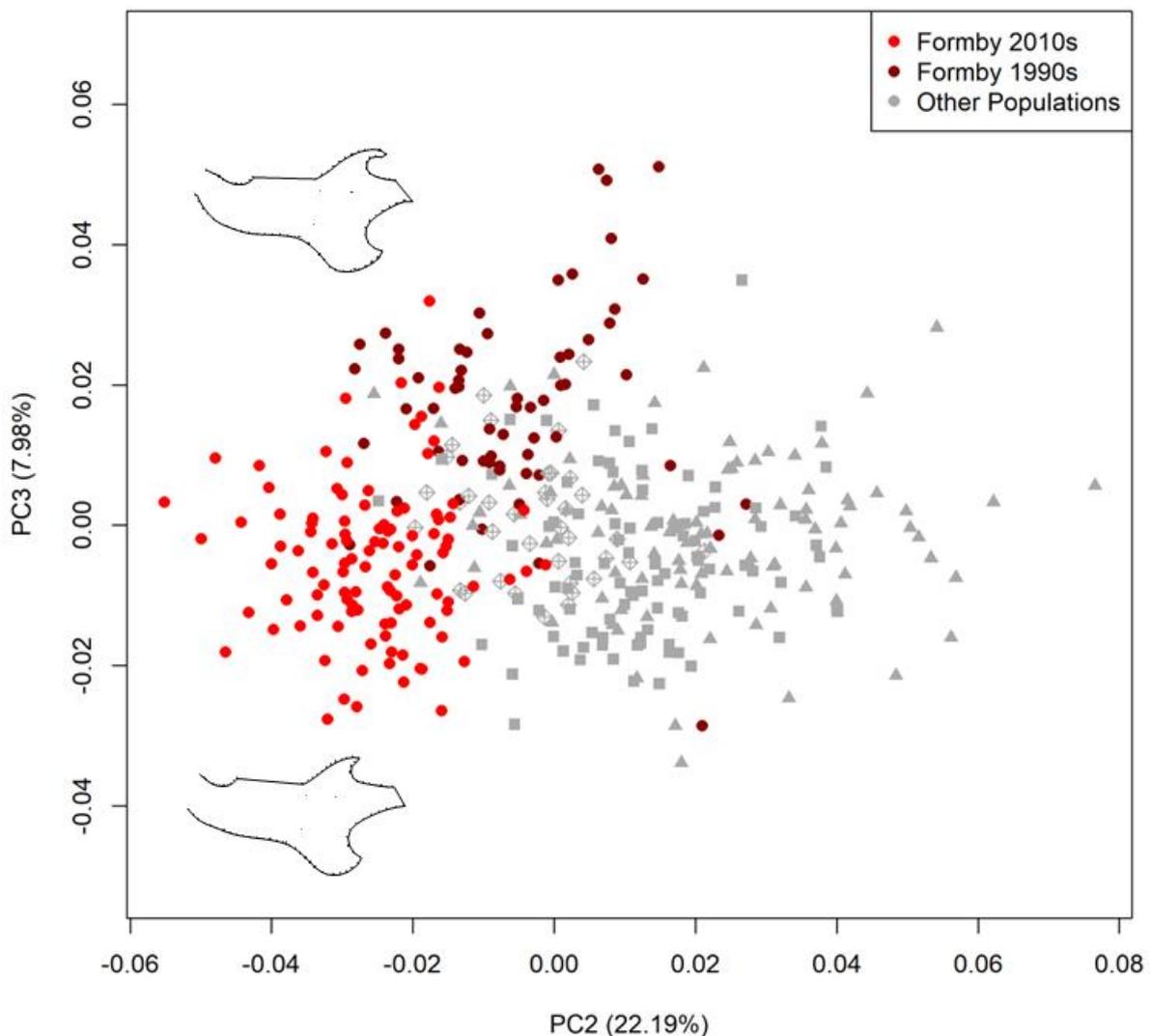


Figure 2.20. Plot of results of principal components analysis of the mandible, showing principal components 2 (responsible for 22.2% of shape variation) and 3 (responsible for 7.9% of shape variation). Each data point represents the shape data associated with a single specimen.

There is a clear split of Formby specimens between decades collected along PC 3 (see Figure 2.20), with 1990s specimens occupying the negative end of the axis and 2010s specimens occupying the positive end. As such, 2010s specimens from Formby are more likely to have a less curved coronoid process and a shorter diastema while 1990s specimens from Formby are more likely to have a highly curved coronoid process and longer diastema.

2.3.9 - Mechanical Advantage Analysis of Mandible

A significant variation was detected in the MA of both temporalis in-levers (Td: $F = 13.725$, $p > 0.001$; Tv: $F = 17.93$, $p > 0.0001$). A post-hoc Tukey test revealed that the Formby population has significantly different Tv and Td MA than both North Scotland and Borders populations ($p < 0.01$, $p < 0.001$). Jersey also showed significant differences in Tv MA with North Scotland ($p = 0.02$) and differences in Tv and Td MA with Borders ($p = 0.015$, $p < 0.01$) but no difference with Formby ($p = 0.85$, $p = 0.89$). Formby specimens had the lowest mean MA for both Tv and Td of all populations, although closely followed by Jersey (see Figure 2.21). No significant difference in MA was detected between decades but analysis of the Formby subset detected a difference in Td ($F = 42.305$, $p < 0.01$), with specimens from 2010 have higher MA than 1990s specimens (see Figure 2.22). According to a Pearson's product-moment correlation coefficient test, no correlation was detected between centroid size and MA of Tv ($df = 3286$, $p = 0.2729$), suggesting MA of Tv is predicted by shape only. No significant difference in any measurement for MA was detected between sexes.

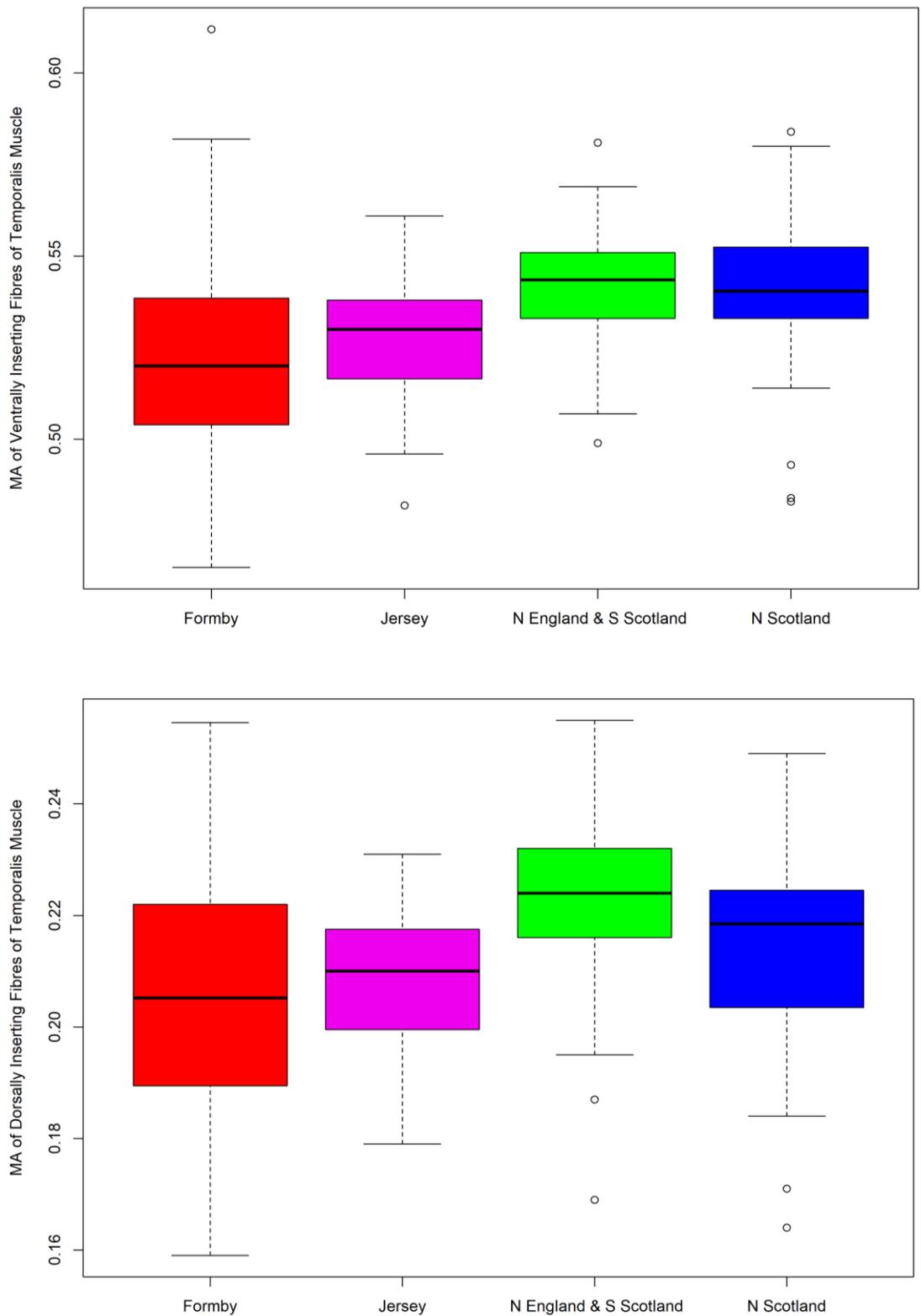


Figure 2.21. Boxplot of mechanical advantage of ventrally and dorsally inserting fibres of temporalis muscles in red squirrel population. Black line represents median, box represents interquartile range and open circles represent data points lying further than 1.5 times the interquartile range.

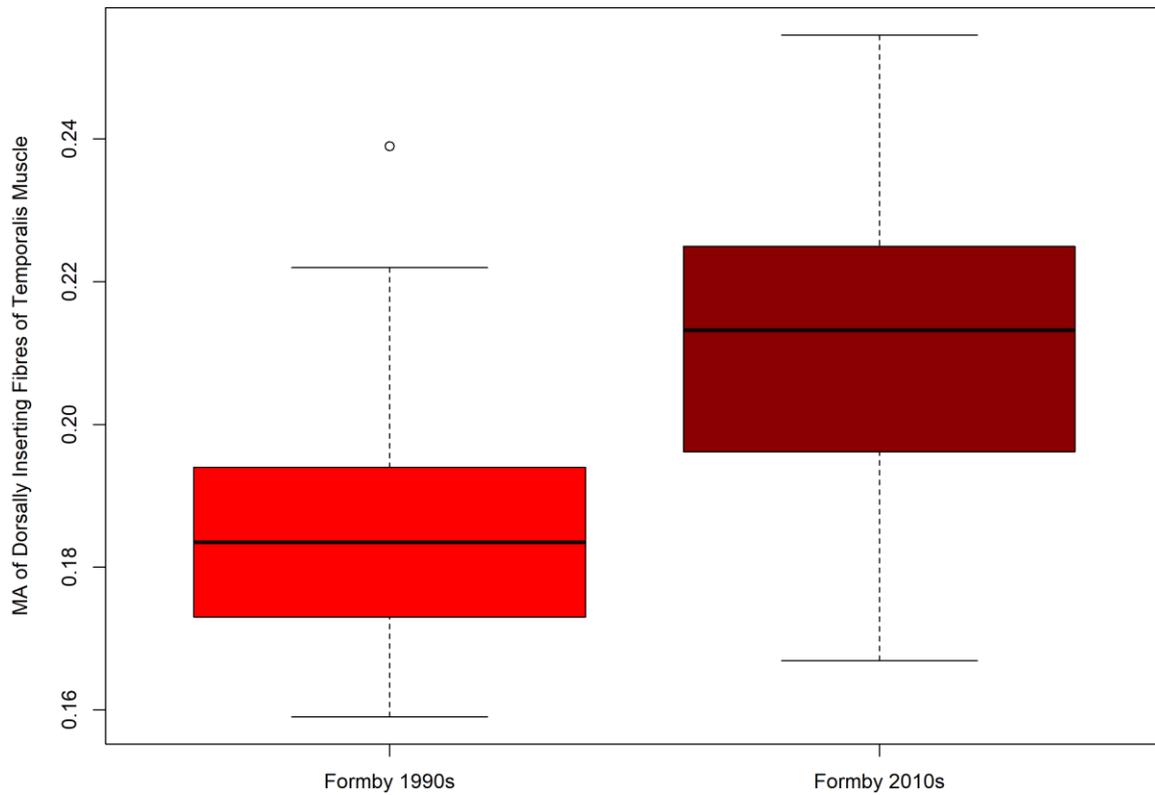


Figure 2.22. Boxplot of mechanical advantage of dorsally inserting fibres of temporalis muscles in Formby red squirrels over time. Black line represents median, box represents interquartile range and open circles represent data points lying further than 1.5 times the interquartile range.

2.4 Discussion

The results of this study show that there is significant morphological variation in the crania and mandible in British populations of red squirrels *Sciurus vulgaris*. The first hypothesis, that there is significant morphological variation between populations across Britain, is supported through Procrustes ANOVA and principal components analysis. There are statistically significant differences in cranial morphology between North Scottish, Borders and Formby specimens, evident in PCAs along PC1, PC2 and PC3. Statistically significant differences in mandibular morphology is also seen in North Scottish, Borders, Formby and Jersey populations, evident in PCAs along PC1, PC2 and PC3.

The second hypothesis, which predicted detectable morphological change within individual populations over time, is only supported here in some populations. Within the Formby subsample we see a distinct change in morphology between specimens collected in the 1990s and specimens collected in the 2010s. This is not seen in the other populations of North Scotland, Borders or Jersey.

The third hypothesis, that the difference in morphological variation has an effect on mechanical advantage of the muscles of mastication is also supported through ANOVA. Formby specimens were shown to have significantly lower MA of both the dorsally and ventrally inserting fibres of the temporalis muscle than other populations. Additionally, Jersey specimens were shown to have significantly lower MA of the ventral temporalis fibres than other populations, except Formby.

2.4.1 - Morpho-Functional Variation between Populations – North Scotland

North Scottish specimens have a cranial length and height intermediate to that of Northern English and Formby specimens but with a dorsally positioned temporalis scar (suggesting a relatively larger temporalis muscle). Their mandibles are generally deep with less well-defined coronoid processes and a deep inflection between the condylar and angular process, contrasting morphologically to Formby specimens. The morphology of North Scottish specimens is likely to affect the functional abilities of the masticatory apparatus, the overall increased size of the temporalis muscle being a unique feature of this population.

Additionally, the ‘blunter’ coronoid process of North Scottish populations increases the distance between the condyle and the coronoid process, which allows for a larger MA of the dorsally inserting temporalis muscles. North Scottish specimens are also unique in their separate allometric trajectories. Analysis of allometric trajectories on the mandible show that

all populations experience a reduction of the angle between the coronoid process and condyle with increasing size, except for specimens in North Scotland. North Scottish specimens retain a relatively short, blunt coronoid process despite an increase in size. This suggests that the North Scottish specimens are unique in that they would theoretically retain a high MA of the temporalis muscle at the upper end of their size range.

2.4.2 - Morpho-Functional Variation between Populations – Borders

Borders specimens have short skulls, high cranial vaults and a ventrally positioned temporalis scar. Their mandibles are generally deep with less well-defined coronoid processes and a deep inflection between the condylar and angular processes, again contrasting morphologically to Formby specimens. No size or shape variation was detected through Procrustes ANOVA between the 1990s and 2000s; however this was analysed with relatively unbalanced population sizes (n=78, n=12). The morphology of northern English specimens would suggest that they have moderately high bite forces, with larger areas for muscle attachment on their high cranial vaults and a high MA of the temporalis muscles supported by mandibular morphology. However, this population has the smallest body size of the dataset which would may affect their overall masticatory abilities.

2.4.3 - Morpho-Functional Variation between Populations – Jersey

Only mandibular specimens from Jersey could be included in this study as the number of intact crania were too few to perform robust statistical analyses. Specimens from Jersey overlap in the morphospace with the Borders and Formby populations, which is somewhat unexpected considering how geographically distant this population is to others. They are overall the largest of the populations studied, and have mandibular morphology most similar to that of the Formby population although with a less pronounced curvature of the coronoid process and somewhat deeper mandible. This affects the mechanical advantage measurements, showing similarly low MA of the dorsal and ventral temporalis fibres as the Formby population. While size may mitigate some of the effects of a low MA, the lack of relationship between overall mandible size and MA of the temporalis muscle suggests an overall lower bite force of this population.

2.4.4 - Morpho-Functional Variation between Populations – Formby

Formby specimens are the most distinct, plotting fairly separate to other populations in both cranial and mandibular analyses. These specimens generally have longer skulls, a flatter

cranial vault, a ventrally positioned temporalis scar, a narrow mandible with a curved coronoid process and shallow inflection between the condylar and angular processes. The flatter cranial vault coupled with a more ventrally positioned temporalis scar suggests an overall smaller temporalis muscle. In the mandible, the angle between the coronoid process and the condyle is more acute resulting in shorter ventral temporalis muscles fibres. This directly affects the mechanical advantage of this area of muscle, leading to the difference in mechanical advantage between populations detected. Like Jersey specimens, Formby specimens are reasonably large, which may go some way to mitigate the effects of a low MA but again, like in Jersey specimens, no relationship between mandibular size and MA of the temporalis muscle is supported. This reduction of size of temporalis muscle may mean a lower overall bite force, or efficiency of the masticatory apparatus, suggesting Formby squirrels may be less well adapted to eating a diet of very hard foods than other populations.

A distinct difference in morphology within Formby specimens is detected in the zygomatic arch, with the unexpected support of the existence of sexual dimorphism. Rodents in general are not sexually dimorphic. Males in this subset are more likely to have zygomatic arches that are positioned closer to the dorsal midline while females have arches positioned more laterally. This follows some of the morphological variation seen between Formby specimens and other populations with males within Formby being more similar to that of other populations than to females. Further analysis only detects this dimorphism with specimens from 2010 onwards, however this is by far the larger group (48 specimens to 13 specimens in the 1990s group), so this may be a result of too small a subset to perform effective statistical analysis. In squirrels, the zygomatic arch is a support system for the masseteric muscles, serving as the point of origin of the posterior deep masseter and zygomaticomandibularis and attachment for the superficial masseter (Cox & Jeffery, 2011). This means this rather slender rod of bone experiences high levels of strain (Cox et al., 2012) and is therefore likely to be susceptible to modification under varying levels of masticatory pressure. The more laterally flared zygomatic arch seen in Formby specimens, and in particular female specimens of this subgroup, may be an adaptation towards different dietary pressures. This would suggest that Formby specimens may have slightly larger masseter muscles than other populations, with females from Formby having the largest. The width of the zygomatic arch where it fully diverges from the cranium, would be another indicator of muscle attachment area, however this area of the cranium is particularly difficult to capture accurately using a 3D surface scanner, due to its slender nature, so could not be measured in this study.

Formby was the only population in which morphological variation was detected over time. Specimens earlier than 2010 show a unique highly curved coronoid process and long diastema while specimens after 2010 show a blunter coronoid process and shorter diastema (more similar to that of North Scottish specimens). It appears that this change in shape is accompanied by a change in size, with more recent Formby specimens being significantly smaller than specimens from 1990s. This contrasts with previous analysis of mandible shape and size (Cox et al., 2020) which showed Formby specimens to be the largest, followed by Jersey. Cox et al. (2020) did not include any specimens from 2010 onwards. With re-analysis including these more recent specimens, Formby mandibles no longer plot as largest, although do show the largest range in size. When looking at crania, Formby specimens still plot as the largest, followed by Jersey. Significant difference in size is detected between year groups, with more recent specimens again being smaller than 1990s specimens, although in reality this difference is minute. Cranial shape varies between the 1990s and 2010s specimens with more recent specimens plotting somewhat further away from the rest of the distribution than 1990s specimens (see Figure 2.14.) while still retaining the typical Formby morphology of a flatter cranial vault, relatively smaller orbit and longer skull. This suggests a trend within the Formby population where the cranial morphology continues to become more distinct from other British populations of red squirrels while the mandibular morphology, as well as size, has regressed somewhat to be more like that of other populations. Effect on functional ability is detectable, with the MA of the dorsally inserting temporalis fibres being significantly lower in 1990s specimens than 2010s. This suggests that the main morphological differences between the decades, the distinctively reduced angle between the condyle and the coronoid process in the 1990s specimens, has reduced the MA of dorsally inserting temporalis muscle fibres, effecting the overall efficiency of muscle output force in 1990s Formby specimens.

For analysis of change over time, specimens were sorted into decades collected, to ensure enough specimens per group for effective statistical analysis, and ease of comparison. Of course, the division of specimens into decades is arbitrary, with many specimens likely to have been born in one decade but died in another. This is likely the case in the North Scotland sample where specimens were collected in three consecutive decades, 1980s, 1990s and 2000s. Additionally, comparison between groups is difficult as not all populations had specimens collected in the same time periods, with differing abundances per decade collected. As such, the detectable morphological change in Formby may have been more obvious considering the groups analysed had 10 + years between them, which may be enough

time for obvious morphological change to arise. With the lifespan of a red squirrel in the wild less than 6 years, the gap between the two time periods allows enough time for individuals born in 1990s to have died before the 2010s.

2.4.5 – Drivers of Morphological Variation – Genetic History

The founder effect may play some role in the morpho-functional distinctiveness of the Formby and Jersey populations, due to their differing genetic histories. The population in Jersey is thought to trace its founders to individuals introduced to Jersey around 1885 (Shorten, 1954) from both southern England and France (Le Sueur, 1976; Magris & Gurnell, 2002). This was later supported through genetic analysis (Simpson et al., 2013). The Formby population is thought to originate from an introduction of squirrels from Scandinavia to Ainsdale (near Southport) in the 1930s (Gurnell & Pepper, 1993; Cox et al., 2020). The population of Cumbria was thought to have the most distinctly ‘British’ genotype, with no known introductions to this population from mainland Europe (Macpherson, 1892; Hale & Lurz, 2003). However, migration across the Kielder forest post 1980 from Northumberland may have helped to widely disperse ‘Scandinavian’ haplotypes that existed in the north-eastern populations. Whether these Scandinavian haplotypes exist due to having a selective advantage or due to recent (last 60 years) introductions from Scandinavia is unknown (Hale et al., 2001, 2004; Hale & Lurz, 2003). The genetic origin of North Scottish populations is also unclear as they suffered a serious decline in numbers in 17th and 18th centuries, with some sources suggesting they may have become entirely extinct in this time period (Shorten, 1954). This was followed by reintroduction projects between 1772-1872 from England and Wales and possibly from Norway or Sweden (Barratt et al., 1999; Shorten, 1954). The presence of Scandinavian haplotypes in the Scottish and Northern English populations may convey a competitive advantage in adaptation to pine forests, however this potential phenotype is not present within the Formby population which also has evidence of Scandinavian introductions. Regardless, the frequent translocations across Britain and from mainland Europe over the last 200 years leads to a muddy knowledge of the genetic history of British red squirrels.

2.4.6 – Drivers of Morphological Variation – Inbreeding

Inbreeding may be another factor affecting the morpho-functional variation within British red squirrels. Formby in particular is likely to have experienced some inbreeding, due to its relative distance from other populations limiting chances for genetic dispersal. Formby has

also experienced a number of population crashes, contributing to likelihood of genetic bottlenecks forming, due to outbreaks of squirrelpox virus. However, these outbreaks occurred in 2008 (Chantry et al., 2014) and again in 2020 (pers. comm K. Hamill). The 2008 population crash, where the population declined by 87% (Chantry et al., 2014), affected the numbers of specimens collected in the 2000s and may go some way to explain the difference in morphology between the 1990s Formby specimens and the 2010s specimens. However, the 1990s specimens are the ones that are the most distinctly different to other British populations, which pre-date any recorded cause for genetic bottlenecking, other than effects of founder effect from the initial introductory population.

2.4.7 – Drivers of Morphological Variation – Diet and Supplementary Feeding

Another factor that may contribute to the morpho-functional variation is differing diets. As discussed previously, the mandible of rodents is highly adaptable to changes in diet, with studies showing that bone remodelling can occur within the lifetime of the animal (Anderson et al., 2014) but also through relatively rapid evolutionary change (Mezey et al., 2000). Red squirrels mainly feed on nuts and seeds with conifer seeds being the most important in the diet (Kenward & Holm, 1993), but deciduous species also eaten such as hazelnuts *Corylus avellana*, beech nuts *Fagus sylvatica*, acorns *Quercus sp.* and sweet chestnut *Castanea sativa* (Tonkin, 1983; Moller, 1986; Magris & Gurnell, 2002). The different populations discussed occupy somewhat differing habitats and therefore have access to different food items.

Red squirrels in North Scotland inhabit mainly pine forests, some of which are plantations of introduced tree species such as Norway spruce but also native pine species such as Scots pine. Red squirrels in North Scotland consume relatively high quantities of scots pine among other pines seeds (Tittensor, 1970; Moller, 1986). Pine seeds develop on the upper surface of the seed-bearing scales of cones. This requires red squirrels to strip off the scales through a mix of gnawing the base off the scale and gripping the scale between their teeth and pulling the scale off. This presumably requires a reasonably efficient gnawing system and a strong bite force, which may explain why the MA of North Scottish red squirrels is higher than other populations that likely consume less pine seeds. It may also go some way in explaining the need to retain robust, deep mandibles in increasing size. If this is accurate, then this may also explain the also high MA of the Borders specimens. This part of the country has a mix of both pine and deciduous tree species, presumably the diet of red squirrels in this area is high in pine seed but also mechanically demanding nuts such as hazel and acorns. Much of this

area is covered by the Kielder Forest which was planted for timber production in the 1980s and consists of largely Sitka spruce *Picea sitchensis*, Norway spruce *Picea abies* and lodgepole pine *Pinus contorta* (Hale & Lurz, 2003). Both populations require high MA that would convey a competitive advantage in a diet rich in pine seed. Indeed, Hale and Lurz 2003 showed some differences in cranial shape between specimens from Cumbria pre and post establishment of the Kielder Forest, namely an increased snout length. The change in diet may a contributing factor of this morphological change. Unfortunately, no specimens prior to the 1980s establishment of the Kielder forest could be included in our dataset, so any morphological differences pre and post merging of the Cumbrian and Northumberland populations could not be assessed.

The island of Jersey is heavily population and most of the island is urbanised, despite this there is some woodland cover, with squirrels feeding on autumn seeds (*Quercus robur* and *Castanea sativa*), with some summer conifer cones (Magris & Gurnell, 2002). It is also noted by Magris and Gurnell that supplementary feeding in gardens by residents of the island is quite common. Formby is also a mixed woodland with native Scots pine, some Corsican pine and broadleaf trees (Gurnell & Pepper, 1993). The site is owned and managed by the National Trust, where up until relatively recently, wardens provided supplementary food to the squirrels, mainly in the form of peanuts (Gurnell & Pepper, 1993).

Supplementary feeding as a method for conservation has both positive and negative impacts. Bird populations have shown to benefit through enhanced survival over winter (Jansson et al., 1981) and in fecundity through increased number of eggs or better egg condition (Robb et al., 2008b) but with some unexpected consequences such as affects to behaviour (Strain & Mumme, 1988; Jokimaki et al., 1996; Wilson, 2001; Robb et al., 2008). Congregation of animals at feeding stations can allow for disease transmission (Sorensen et al., 2014; Murray et al., 2016). Such concerns about spread of disease lead to the establishment of the Garden Bird Health Initiative in the UK in 2005 and later, the Garden Wildlife Health Initiative in 2013 (RSPB, 2023). For red squirrels, supplementary feeding can be highly beneficial, providing a buffering affect against the variabilities in natural food availability (Shuttleworth, 2000). Studies in Jersey, show that squirrels that did not go on to produce litters were less likely to have received supplementary food, suggesting that provision of supplementary food can increase fecundity within a population (Magris & Gurnell, 2002). Supplementary food could also allow for increased body size, as individuals squirrels are better nourished as juveniles and able to reach maximum size. However, when done without sufficient research,

negative effects can occur such as nutritional deficiencies or disease spreading at feeding stations, with particular concerns about squirrelpox virus (Shuttleworth, 2000).

This study suggests that supplementary feeding could also have implications for the biomechanical abilities of the masticatory apparatus. Comparably, supplementary foods are often easier to access, either as unshelled nuts or foods that are generally less difficult to access like peanuts. This could mean that in populations that consume large quantities of less mechanically demanding foods, the selection pressure for higher bite forces is removed, resulting in a loss in efficiency of the masticatory apparatus. This may go in some way to explain the reduced MA of the mandible musculature in red squirrels in both Formby (which received large amounts of peanuts) and Jersey (which are provided supplementary food from the public). Although previously suggested by Cox et al. 2020, further evidence supporting this is presented here in the morphology between 1990s and 2010s Formby specimens. 2010s specimens have a MA that is much higher than that of the 1990s specimens, caused by a change in morphology since the 1990s. This change in morphology and function coincides with a change in conservation practices in Formby. Although providing peanuts to visitors was extremely prevalent in the 1990s, this practice is no longer encouraged by the National Trust (A. Brockbank, pers. comm. 2020). In the 1990s 25-57% of the food items eaten by red squirrels in Formby were peanuts (Shuttleworth, 1997; Shuttleworth, 2000). Presumably this proportion has dramatically dropped since then, which may have encouraged an adaptive response of 2010s Formby squirrels to the more mechanically demanding natural diet.

While the relationship between morpho-functional variation in British red squirrels and diet is still tentative, the potential impacts of supplementary feeding may impact future choices of conservation workers, both in choice of supplementary food provided to managed populations, and also in translocation projects. Many conservation strategies in the UK and elsewhere involve taking individuals from a dense population to another location where numbers are low (Ogden et al., 2005; Poole & Lawton, 2009)). There are ethical implications involved in translocating individuals in this manner, with the survival of the individuals in the new habitat is of both ethical importance and a main goal of the conservation project. It may be that individuals that are less equipped to the locally available diet struggle to consume adequate calories to survive the initial first weeks of translocation or to achieve fecundity in the next season (Kenward & Hodder, 1998). Either way, the initial survivability of an individual in the early days after translocation is vital. In terms of provision of supplementary food, the speed of which managed populations adapt morphologically to a provided food

source may inform the importance of continuing provision rather than sudden removal of said food source.

2.4.8 – Conclusion

The conclusion of this study, that differing diets could be a cause of the morpho-functional variation seen in British red squirrel populations, is tempting. However, further information is needed to establish whether diet is indeed the main driver of change. Firstly, it is necessary to establish whether the squirrels in this dataset are indeed consuming the diets we assume from their habitats. Isotope analysis is currently ongoing into specimens within this dataset to establish whether there is a detectable chemical signal within the skeletal material, suggestive of a pronounced difference of diet. Secondly, it is necessary to assess the assumption of this conclusion, that peanuts are less mechanically demanding food items. When accessing the edible parts of a nut or seed, squirrels are faced with physical challenges. Plants have evolved specific physical barriers to protect their seeds from predators, bordering a fine line between total inaccessibility while allowing for the seed to eventually germinate (Lucas, 2004). How difficult these outer casings are to fracture determines the lower limits of biomechanical function necessary for an animal to be able to regularly consume these food items. While we can state that peanuts are a less mechanically resistant food than others, it is helpful to be able to quantify just how different they are from other, natural, food items. As such, it is necessary to gather data on the material properties of the different food items consumed by red squirrels in Britain. Chapter 3 of this thesis will aim to establish toughness of a selection of nuts and seeds with the aim of building a profile of the mechanical difficulties faced by the populations discussed here when accessing food items. Hopefully, results from this will allow a correlation to be seen between the material properties of different food items and the masticatory abilities of the squirrels consuming them.

2.4.9 - Future Work

Additionally, further work is necessary to establish the effect of morphology on functional ability. Mechanical advantage analysis is a helpful method of establishing muscle efficiency of the mandible. However, this method is simplistic. More comprehensive methods can be employed to analyse how differing morphologies of red squirrel mandible and crania perform under loads. An example of this, that may yield interesting results, is finite element analysis. A finite element analysis study of the mandibular anatomy of the populations discussed here

will make up Chapter 4 of this thesis. And finally, an investigation into the genetics of British red squirrel populations may offer us a further insight into the overall story of the differences of red squirrel populations and how they respond to the high levels of fragmentation they have

3. Investigation of the Material Properties of Red Squirrel Food items and the effect of caching.

3.1 Introduction

Squirrels are expert consumers of hard foods, using their incisors to gnaw through the tough outer casings of seeds and nuts. This proficiency has been conveyed through unique adaptations. Sciuromorphy, a morphological condition whereby the masseter muscle extends dorsally onto the rostrum (Druzinsky, 2010a), allows for increased efficiency in generating incisor bite forces compared to other rodents (Casanovas-Vilar & van Dam, 2013; Cox et al., 2012; Druzinsky, 2010b). In addition, like all rodents, they have distinctive oversized incisor teeth that grow continuously through their lives, to combat the continuous wearing down of the teeth through gnawing.

In Britain, red squirrels *Sciurus vulgaris* have suffered a serious decline in numbers due to the introduction of invasive grey squirrels in the late 1800s (Shorten, 1952, 1954). They now persist in a highly fragmented distribution with limited genetic dispersal between populations. These populations occupy varying habitats with access to different food items. In Northern Scotland, most red squirrels occupy woodland consisting of conifer tree species, where they will generally consume the seeds of either native Scots pine *Pinus sylvestris* or introduced species such as Norway spruce *Picea abies* and Sitka spruce *Picea sitchensis* (Moller, 1986; Shuttleworth, 2000; Hale & Lurz, 2003; Gurnell et al., 2004). In Northern England and Southern Scotland, woodland consists of a mix of non-native conifer trees and native deciduous trees providing a diet of pine seed, hazelnuts *Corylus avellane*, sweet chestnuts *Castanea sativa*, beechnuts *Fagus sylvatica* and acorns (*Quercus* sp) (Magris & Gurnell, 2002; Moller, 1983, 1986; Tonkin, 1983). In suburban areas, such as the Isle of Wight, peanuts, *Arachis hypogaea*, in the shell (commonly referred to as monkey nuts) are often provided by the public as wildlife food (Butler, 2021; Wight Squirrel Project and The Isle of Wight Red Squirrel Trust., 2015). Red squirrels in suburban areas therefore regularly consume a non-negligible amount of peanuts. In Formby National Reserve, Merseyside, woodland is mainly Scots pine but squirrels have also had access to uniquely high proportions of peanuts, either through past conservation projects, or provided by visitors to the reserve (Gurnell & Pepper, 1993; Shuttleworth, 2000).

Plants have evolved defensive measures to protect against consumption by granivores. In the above example this is either a hard outer shell (as in the true nuts – hazel, beech, oak) or

fibrous cones (Scots pine, spruce species)(see Figure 3.1). Cones are rigid structures housing numerous seeds, with each seed protected by a scale. Squirrels have become adept at removing the scales, stripping them in a methodical manner from the axis, starting from the top of the cone, moving downwards until most scales are removed (Moller, 1986). Pine seeds are much smaller than other food items in the red squirrel diet, and require more effort to access, but have the highest energy value (6.0-6.8kcal/g) (Grodziński & Sawicka-Kapusta, 1970) and have a high fat content (Sen et al., 2016). True nuts, such as hazel, beech and acorn, encase the edible kernels with a hard outer shell (in the case of both acorns and beechnuts, the nuts are attached to the tree by a soft husk or ‘cupule’ that drops off when the seeds become ripe, see Figure 3.1). While the energy requirements of breaking through the hard outer shells are likely high, the seeds are larger and can provide high calorie rewards (5.9 kcal/g for hazelnuts and beechnuts)(Grodziński & Sawicka-Kapusta, 1970). However with acorns, the calorie reward is lower (4.4-4.9kcal/g) and red squirrels do not appear particularly well adapted to digesting acorn polyphenols (Kenward & Holm, 1993). Peanuts are legumes, and what is commonly referred to as the seed shell, is actually the seed ‘pod’, and is relatively soft compared to true nuts. Nutritionally, peanuts are high in monosaturated fats and protein (Arya et al., 2016) but comparatively low in energy 5.6kcal/g (Brufau et al., 2006).

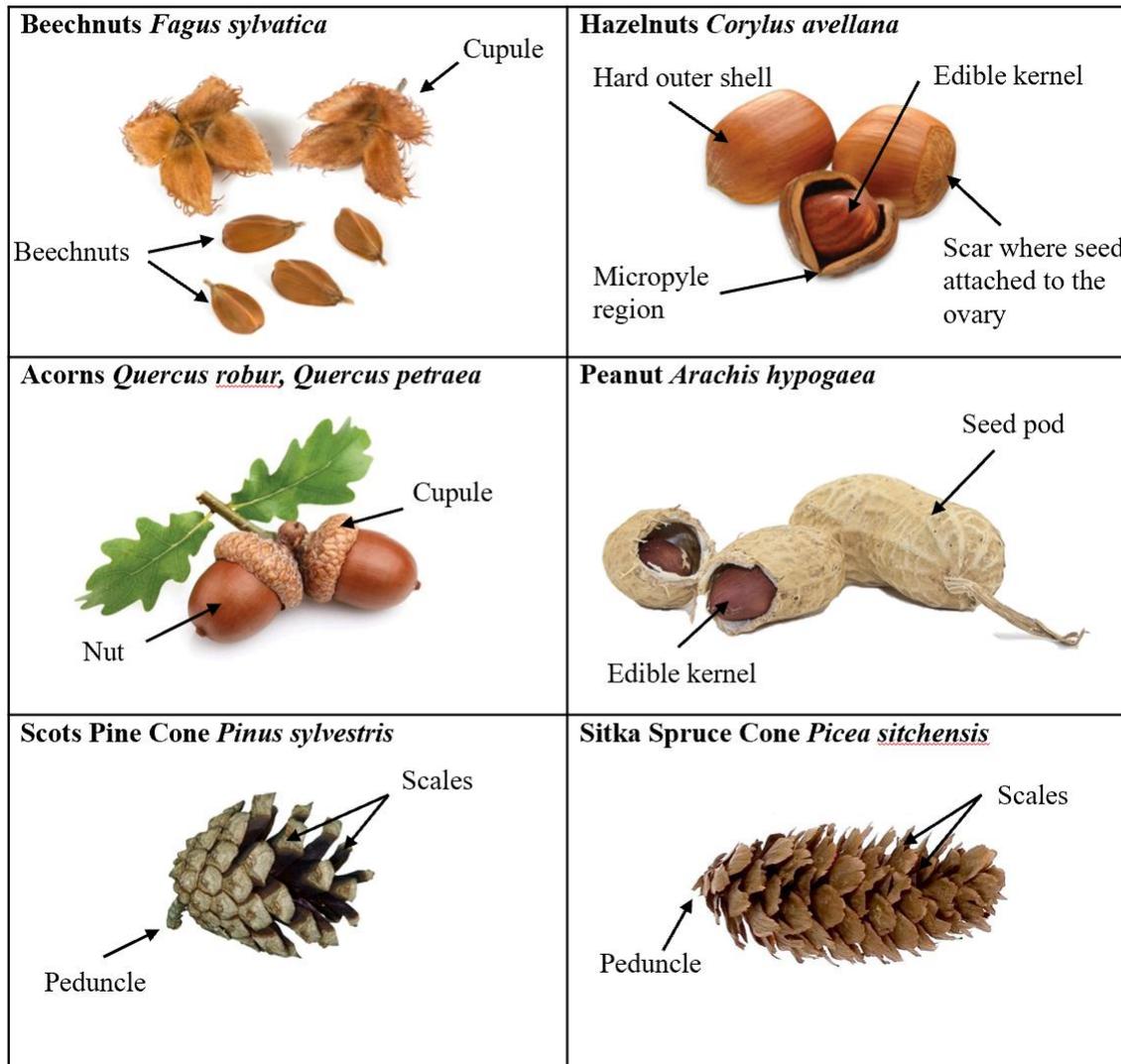


Figure 3.1. Anatomy of the food items regularly consumed by British populations of red squirrels.

To ensure adequate food supply throughout the year, squirrels are among many animals that ‘cache’ food items for later consumption. In particular, red squirrels are known to scatterhoard individual seeds, nuts or cones and return for later retrieval (Wauters et al., 1995). They usually cache items in the autumn, when food supplies are abundant, and recover them throughout the winter (Wauters et al., 1995). Items that are most commonly cached are nuts and cones, which are usually buried in soil or leaf litter at a depth of 2-6cm (Wauters & Casale, 1996; Shuttleworth, 2000). Supplementary food items are also cached. Limited research into the effect of caching on material properties of nut and seed casings/shells has been performed, but the conditions of burial are likely to have an effect, with one study

showing that fracture toughness can be significantly affected by moisture content on the shell of the African mongongo nut (Williamson & Lucas, 1995a).

The range of red squirrel diets across Britain likely pose different mechanical challenges to squirrels, conferring varying requirements of masticatory muscle size in order to generate the necessary bite forces. This likely effects the underlying skeletal morphology. Morphological analysis of red squirrels skeletal anatomy in Cox et al. (2020) and in Chapter 2, shows that, between populations, there is significant shape variation in the hard tissues of the mandible and crania. It is likely that this morphological divergence confers functional abilities and is therefore able to be acted upon by selection. However, the rapid changes in the mandible shape of the Formby population within just a few decades, coinciding with a removal of peanuts as a supplementary food (A. Brockbank, pers. comm. 2020), suggests that the morphological differences in British red squirrels are likely due to plastic changes rather than evolutionary change.

To establish whether these morphological changes are linked to diet, it is important to first establish whether there are significant differences in the material properties of the food items consumed by red squirrels across Britian. Some previous research on the material properties of nuts and seeds exists, however, these are usually limited to one or two food items and usually those consumed by primates (e.g. Lucas et al., 1991; Evans & Sanson, 1998; van Casteren et al., 2019). Macadamia nuts and hazelnuts are a focus of a considerable amount of research, due to their use in the confectionery industry (Jennings & Macmillan, 1986; Lucas et al., 1994; Wang & Mai, 1994; Liu et al., 1999; Ercisli et al., 2011; Kaupp & Naimi-Jamal, 2011).

The methods used to investigate material properties of food items vary between studies. When performing in situ analysis in the field, indentation studies measuring Vickers hardness have been performed with portable devices (Abbott et al., 1977; Lucas et al., 2009). Simpler in-the-field studies have also been done, such as by (Boesch & Boesch, 1983), where ‘hammers’ (rocks and other implements used by chimpanzees) were dropped from varying heights onto nuts, with the height and weight of the ‘hammer’ recorded. However, this comes with a variety of inconsistencies and the results are anecdotal rather than quantitative. In an investigation of niche separation of neotropical bat communities, insect and fruit foods had their mechanical properties investigated through pushing the flat end of a screw (connected to a force transducer) into the food item and measuring the force required to produce

mechanical failure of the external surface (Aguirre et al., 2003). This is a unique technique, but difficult to reproduce.

When experiments can be performed *ex situ* in a laboratory, compression testing appears to be most commonly used (Janzen & Higgins, 1979; Peters & Maguire, 1981; Peters, 1987; Kinzey & Norconk, 1990; Lucas et al., 1991; Williamson & Lucas, 1995a; Liu et al., 1999; Patel et al., 2008; Ercisli et al., 2011; Kacal & Koyuncu, 2017; van Casteren et al., 2019; Hunter, 2021;). Set-up usually includes a rigid plate that slowly applies a load onto an object until mechanical failure occurs. The force required to ‘break’ the item is recorded. The speed at which the load is being applied can be adjusted as well as the minimum drop in resistance, so the point at which mechanical failure occurs can be more accurately recorded.

Additionally, different tools can be connected to the device to mimic differing tooth shapes, either ‘idealistic’ tooth shapes to study theoretical abilities of different teeth (Anderson & LaBarbera, 2008; Anderson, 2009; Crofts & Summers, 2014) or models of real animal teeth (Lucas et al., 1994; Berthaume et al., 2010; Hunter, 2021) to more closely simulate real-world conditions. Ercisli (2011) employed compression testing to examine the mechanical properties of 9 cultivars of hazelnut, obtaining values for ‘rupture force’, ‘deformation’, ‘energy absorbed’ and ‘hardness’ for both the kernel and the nut. Kacal & Koyuncu (2017) focused on best practice for cracking the hazelnut shell, measuring the force, energy, and specific deformation required for initial rupture and kernel extraction (Kacal & Koyuncu, 2017).

To be able to make comparisons of mechanical challenges offered by different food items, it is first necessary to establish measurable parameters that adequately reflect real-world conditions. Many measurements can be investigated to describe how biological objects behave under loading during mastication. Hardness has been used in many studies, often with conflicting definitions between studies. Some studies define hardness as resistance to indentation or plastic deformation (occasionally called Vickers Hardness (Lucas, 2004; Lucas et al., 2012)). Other studies use hardness and toughness interchangeably.

Stiffness is a measure of how an object resists elastic deformation. It can be seen on a stress-strain curve as the initial slope – therefore it is essentially a measure of how much energy is required to initiate a crack in a structure. Materials that have high stiffness, termed brittle, require considerable work to start a crack but once a crack is initiated, catastrophic failure occurs. Toughness, in contrast, is a measure of how well a material resists fracture

propagation (Lucas, 2004). Toughness can therefore be measured as the work done per unit area of the crack being propagated. While stiffness can be a useful material property to consider, especially when studying brittle materials such as seed casings, comparisons between materials are difficult as stiffness values can vary between small and large samples, even of the same material. Toughness measurements incorporate size as part of the calculations and therefore are more useful for comparison between biological objects with natural variability in size. Additionally, toughness is likely more important to consider in the context of red squirrels. Most nut shells are relatively stiff but propagating the initial crack is an important step of opening the shell.

Here, I collect values for three variables that I define as follows: 1 - Energy to initial fracture (mJ), defined as the amount of work done to initiate a crack in a whole, undamaged nut, as output by software through compression tests; 2 – Force to initial fracture (N), defined as the amount of force required to initiate a crack in a whole, undamaged nut, as output by software through compression tests; 3 – Work-of-Fracture or ‘Toughness’, here defined as energy per area cut (see equation below). The energy to initial fracture and force to initial fracture are both variables that are easily collected through compression testing. Additionally, force to initial fracture can be compared between nuts as a commensurate to bite force, while energy to initial fracture is commensurate to the amount of effort actually needed to access the food item. Work-of-fracture is likely the most useful variable for comparison as the relative size of the object is also included in the calculations.

3.1.1 Aims

This study aims to create a profile of the mechanical challenges involved in accessing food items faced by red squirrels across three main British populations. This is with the goal of assessing whether morphological divergence in the masticatory apparatus (as observed in Chapter 2) is potentially linked to a need for higher bite forces as an adaptation to the differing mechanical challenges of their available diet.

3.1.2 Hypotheses

1) *The outer casings of supplementary food items consumed by red squirrels (namely peanuts) have lower work-of-fracture, energy to initial fracture and force to initial fracture than natural items obtained from the wild.* This is dependent on the assumption that the relatively ‘soft’ shell of peanuts would be significantly easier for a squirrel to bite through than wild food items such as hazelnut shells, acorns shells or pine cone scales. Values of

these measures will be obtained through compression tests and compared across food item types.

2) *Caching will reduce the toughness, energy to initial fracture and force to initial fracture required to break the shells (or cone scales) of red squirrel food items.* This is due to changes in environmental factors including time removed from the tree and moisture content, as shown to have an effect on mechanical properties of nuts shells in Williamson & Lucas (1995b).

3) *Squirrels in Formby National Reserve, Merseyside face reduced mechanical challenges in accessing adequate nutrition than squirrels in Northern England and Scotland.* This is due to the high proportion of peanuts consumed by squirrels in Formby, which likely have lower toughness and energy/bite force requirements to access the edible portion of the nut.

3.2 Materials and Methods

Food items which red squirrels regularly consume were identified from the literature (Shorten, 1954; Tonkin, 1983; Kenward & Holm, 1993; Shuttleworth, 1997; Kenward et al., 1998; Gurnell et al., 2004; Shuttleworth et al., 2015; Butler, 2021). Samples of these food items were sourced from either commercial sources, hand collected by myself or were collected as part of a Citizen Science project, funded by a Royal Society APEX public engagement grant awarded to Dr Philip Cox (see details of source of food items in Appendix 2). As part of this project, material was generated to aid in collecting the correct samples and distributed to the schools prior to collection dates (see examples in Appendix 3). Participating schools collected food items in local woodland habitats and posted them to the University of York for materials testing. Three schools participated in collection of food items – these were Liscard Primary who collected material in the Wirral, Merseyside, Yarrells School in Poole, Hampshire that collected material from Brownsea Island and Bledlow Ridge School in Buckinghamshire.

3.2.2 Physical testing - Whole Nuts

Hazelnuts, peanuts, beechnuts and acorns were tested for material properties both as the whole nut, in its shell, and with smaller cut samples from the shell. A flat plate was attached to the mobile element of a universal testing machine (Mecmesin MultiTest 2.5~i) fitted with a 2.5 kN load cell. For ease of experimental design the whole nuts were placed on the platform, positioned directly under the mobile element, and allowed to rest wherever they

settled naturally. This meant, for all specimens, the load applied was directly applied to the sides of the item, between the micropyle region and where the nut was originally attached to the ovary/cupule (see Figure 3.1 for details of nut anatomy). Displacement was set to increase at a rate of 5mm per minute, and all data was reset once resistance was met (the flat plate contacted the food item). This was then set to apply load at a steady rate until either a pre-set displacement was reached or a percentage break of 50% or higher occurred. The displacement rate was modified according to the item tested, due to varying object size, shell thickness and shell flexibility (see Table 3.1). Maximum displacement (mm), maximum overall force applied (N) and work done/energy (mJ) was recorded for each sample. In order to select appropriate displacement and percentage fracture per food item, a pilot test was conducted where different limits were trialled. The lowest limits required to consistently create a visible fracture larger than 5mm, that penetrated through the width of the shell (so access to the kernel was obtained) were selected. The values can be seen in Table 3.1 below.

Table 3.1. Table showing the displacement and percentage fracture set for compression tests of whole nuts.

Nut Type (Whole Nut)	Displacement (mm)	Percentage Fracture
Hazelnuts <i>Corylus avellane</i>	4.0 mm	50%
Peanuts <i>Arachis hypogaea</i>	4.0 mm	80%
Beechnuts <i>Fagus sylvatica</i>	2.8 mm	50%
English Oak Acorns <i>Quercus robur</i>	2.8 mm	50%
Sessile Oak Acorns <i>Quercus petraea</i>	2.8 mm	50%

3.2.2 Physical testing: Shell Segments

Fracture toughness is difficult to measure in biological tissues as the crack may blunt or deflect easily as it follows the path of least resistance in the structure (Vincent, 1990). To circumvent this Atkins & Mai (1979), and later Atkins & Vincent (1984), developed tests using sharp blades in a guillotine structure to cut across the whole width of the material being tested. Lucas & Pereira (1990) used tailoring scissors as the blade. Here I used a nail clippers in substitute for scissors as the narrow blade of scissors were likely to experience damage before effectively fracturing the shells of the more difficult nuts (e.g hazelnuts or acorns). Nail clippers were secured to the base plate of the testing machine using a cold-curing methylmetacrylate-based glue designed for strain gauges (X60, HBM UK Ltd.) as described in Bonser et al. (2004) and Farran et al. (2008) (see Figure 3.2). The blades of nail clippers meet rather than passing each other, preventing movement/bending of the food item. Prior to use in the study, the lever that depresses the upper blade of the clippers was removed so force could be directly applied to the upper blade. The blades of nail clippers are often curved, but nail clippers that were relatively straight were selected for this experiment. Samples of less than 6 mm in width were cut from the shells/scales of food items, to avoid much of the curvature. For the true nuts included in this study (hazelnuts, beechnuts and acorns) shell

segments were taken from the main body of the nut shell, avoiding the areas where the micropyle penetrates the seed coat and the scar where the seed attached to the ovary. Segments were taken from the main body of the peanut seed pod, avoiding the apex where the pod was attached to the wider plant. For cones of sitka spruce, Norway spruce and scots pine, scales were removed from the cone at random and were cut with sharp scissors into uniform square shapes. Pieces of shells were also filed down to a uniform shape using high grit sandpaper if necessary. Samples were cut both perpendicular and parallel to the growth axis. Prior to compression, the width, thickness and direction of growth of each sample was recorded. Displacement was set as the distance between the blades (3.31 mm) to ensure a clean cut across the width of the sample. For each sample, after testing, the energy (mJ) required to close the clippers without a sample was recorded. This was done after every tenth test, as some damage to the clippers through repeated tests was expected. These energy values were then subtracted from the previous values to calculate the work required to fracture the sample, without including the work required to close the clippers.



Figure 3.2. Image of nail clipper testing set-up. Here the flat plate meets the nail clippers and depresses the top blade (acting as the lever would in normal use). The sample is placed between the two blades in the central 6mm of the clippers where the curvature of the blades is less pronounced.

Values for ‘work-of-fracture’ were then calculated as the work done divided by the cross-sectional area of the cut. As the experimental procedure was set so the blades of the clippers would close completely, a clean cut across the width of the sample was made. Therefore, the width of the sample multiplied by the thickness of the sample is the cross-sectional area of the cut.

$$\textit{Work of Fracture} = \frac{\textit{energy to cut sample (mJ)}}{\textit{cross sectional area of cut (mm)}}$$

3.2.1 Caching Experiments

Nuts and seeds that are hand collected can vary in quality, as the time the item has been removed from the tree, and the conditions that the item has experienced since removal, are generally unknown. To ensure consistency in the caching experiments, the samples used were mainly sourced from commercial suppliers of tree seeds, for floristry or wildlife food. Where suppliers of specific nuts or cones could not be found, samples of acorns of *Quercus robur* and beech nuts (*Fagus sylvatica*) were collected from a single location at a single date on the University of York campus. Purchased and collected samples were ‘cached’ within one week of receiving the delivery. Samples collected as part of the citizen science project were tested within one week of receiving the delivery.

Whole nuts with no obvious damage to the shells were selected for the caching experiments. Samples were ‘cached’ in plastic containers of uniform size - either 18 x 12 x 6 cm for hazelnuts, peanuts, beech nuts, acorns and chestnuts and 17.5 x 11.8 x 8.3 cm for cones. Approximately 10 items were stored per container, however often this was fewer due to limitations on number of samples per nut that could be sourced. Samples were subjected to three different substrate conditions: stored in empty containers, buried in dry construction sand or buried in damp construction sand. The sand was dried in an oven at 150°C for 30 minutes and cooled before use in the experiment to ensure a totally dry and sterilised substrate. Containers with no sand or dry sand were sealed with silica gel packets, to maintain humidity levels. Damp sand was created as ten parts dry sand to one part deionised water. Containers of ‘buried’ samples contained 400g of sand (for smaller containers) or 600g (for larger containers) to ensure samples were buried at a depth of at least 3cm (roughly representative of how deep food items are cached in soil by squirrels (Wauters & Casale,

1996)). All containers were sealed to prevent moisture escaping. Samples were either stored for 1 week, 3 weeks or 3 months prior to physical testing. Samples were stored in two separate temperature conditions, in a refrigerator at a temperature of 3-5 °C and in a temperature-controlled room at 12 °C. This resulted in a total of 18 experimental conditions per food item type plus a control sample.

3.2.3 Wild Samples

Samples collected through the citizen science project were categorised separately as “wild” samples. These were collected throughout the year in specific locations. The majority of “wild” samples collected were Scots pine or Norway spruce. A few specimens of English acorn and hazelnut were also gathered but as these numbered just one or two specimens they were removed from the analysis.

3.2.4 Statistical Analysis

Data from the compression tests were collected using the software associated with Mecmesin’s physical tester, Emperor(version 1.18-408) and transferred to Excel. All statistical analyses were performed in the R statistical environment (R Core Team, 2023).

Analysis was performed on two datasets: the whole nut dataset, in which data was collected from whole nuts under compression; and the work-of-fracture/toughness dataset in which data was collected from shell fragments cut with nail clippers. Values of work-of-fracture/toughness (mJ/mm), energy to initial fracture (mJ) and force to initial fracture (N) were compared across all nut samples to establish which nuts conveyed the most challenge to squirrels. Further analysis of caching experiments compared values of work-of-fracture/toughness, energy to initial fracture and force to initial fracture between different caching conditions within single food item types. Normality in the dataset was tested visually and with Shapiro-Wilk’s tests. Results of normality tests were then used to inform whether parametric or non-parametric tests must be used. Kruskal-Wallis tests were chosen as a non-parametric alternative for analysis of variance. Significant results of Kruskal-Wallis prompted use of post hoc pairwise comparisons, for which Wilcoxon rank sum tests (otherwise known as the Mann-Whitney U test) were employed. Three-way analysis of variance tests (ANOVA) were also performed to analyse the effect of caching variables on work-of-fracture, energy to initial fracture and force to initial fracture. A p-value of less than 0.5 is taken as a significant result. For visual comparisons across groups, boxplots were made in base R.

3.3 Results

3.3.1 All Species: Work-of-fracture/toughness

Initial analysis of work-of-fracture/toughness of uncached samples showed significant differences ($H = 55.6$, $df = 6$, $p < 0.00001$) between species (see Figure 3.3). Pairwise Wilcoxon tests showed the mean work-of-fracture required of English oak (*Quercus robur*) acorn shells was significantly higher than all other species, except sessile oak (*Quercus petraea*). Sessile oak acorn shells required significantly higher work-of-fracture values than all other species, except for beechnuts (*Fagus sylvatica*). Despite the wide range of work-of-fracture values of beechnuts, they were only detected to be significantly higher than peanuts ($p = 0.041$) and Scots pine ($p = 0.024$). Peanuts (*Arachis hypogaea*) had the lowest mean work-of-fracture (see Table 3.2) of all species but no significant difference was detected between peanuts and hazelnuts (*Corylus avellana*), Scots pine (*Pinus sylvestris*) or Sitka spruce (*Picea sitchensis*).

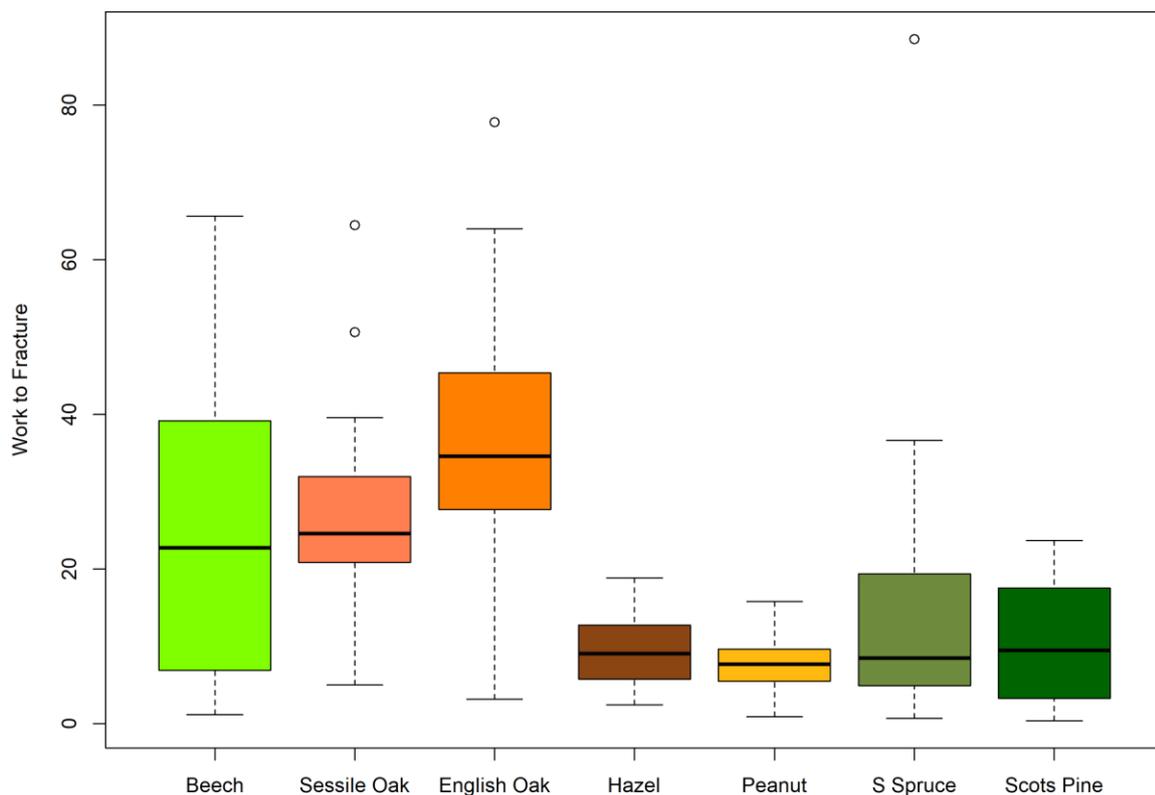


Figure 3.3. Boxplots showing work-of-fracture values of shell/scale segments of food items, cut in experimental procedure described in section 3.2.2. The black line represents the median, the box represents the interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.

An analysis of all species with both uncached and cached samples was performed (where all samples experiencing any form of caching were labelled as ‘cached’). This detected significant differences in mean work-of-fracture of all groups (Kruskal-Wallis: $H = 440.5$, $p < 0.0001$). Pairwise Wilcoxon tests detected significant differences between most groups. Samples of uncached peanut shells required the lowest mean work-of-fracture (7.6 mJ/mm) and cached beechnuts required the highest (74.6 mJ/mm)(see Figure 3.4). However cached beechnuts proved a significant outlier with most samples requiring mean work-of-fracture between 7.6 and 36.4 mJ/mm. Of all samples, those that showed significant difference in work-of-fracture between cached and uncached samples were beechnuts ($p < 0.0002$), sessile oak acorns ($p = 0.022$), hazelnuts ($p = 0.003$) and peanuts ($p = 0.0006$).

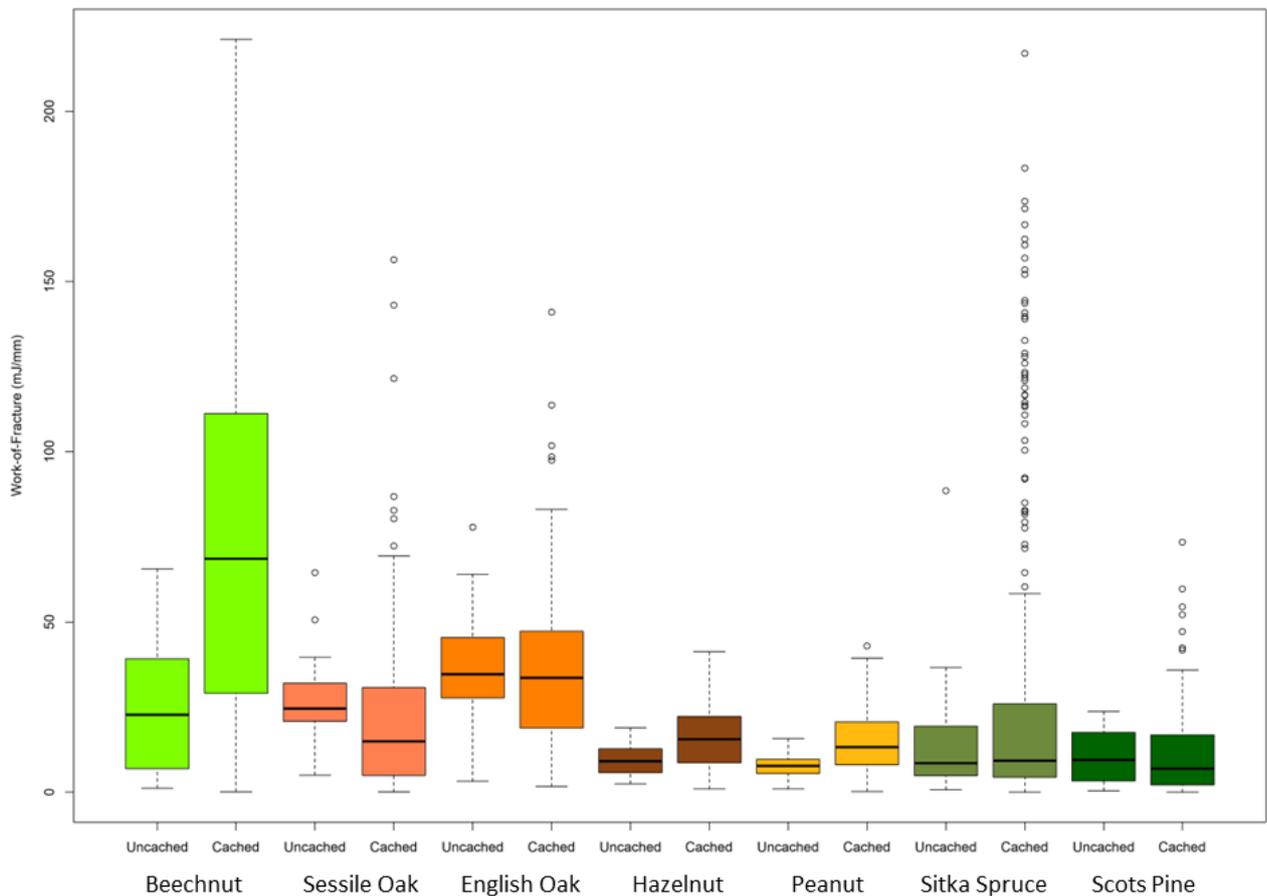


Figure 3.4. Boxplots showing work-of-fracture values of shell/scale segments of food items, both uncached and cached samples, cut in experimental procedure described in section 3.2.2. Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.

3.3.2 All Species: Energy and force to initial fracture

A significant difference in energy to initial fracture was detected between whole uncached samples (Kruskal-Wallis: $H = 474.1$, $p < 0.001$) (see Figure 3.5). Beechnuts had the lowest energy to fracture at a mean of 81.34 mJ. This was followed by peanuts with a mean of 135.67 mJ. English oak acorns had the highest mean of 353.1 mJ. Differences between all species were all shown to be significant through pairwise Wilcoxon tests.

Similarly, a significant difference in force to initial fracture of whole uncached samples was detected (Kruskal-Wallis: $H = 663.3$, $p < 0.001$) (see Figure 3.6). Beechnuts again required the lowest mean force to fracture (55.9 N), however this was not significantly different to the mean force to fracture of peanuts (57.8 N), as detected by pairwise Wilcoxon tests ($p = 0.072$). An additional difference was that hazelnuts required the highest mean force to fracture (320.1 N).

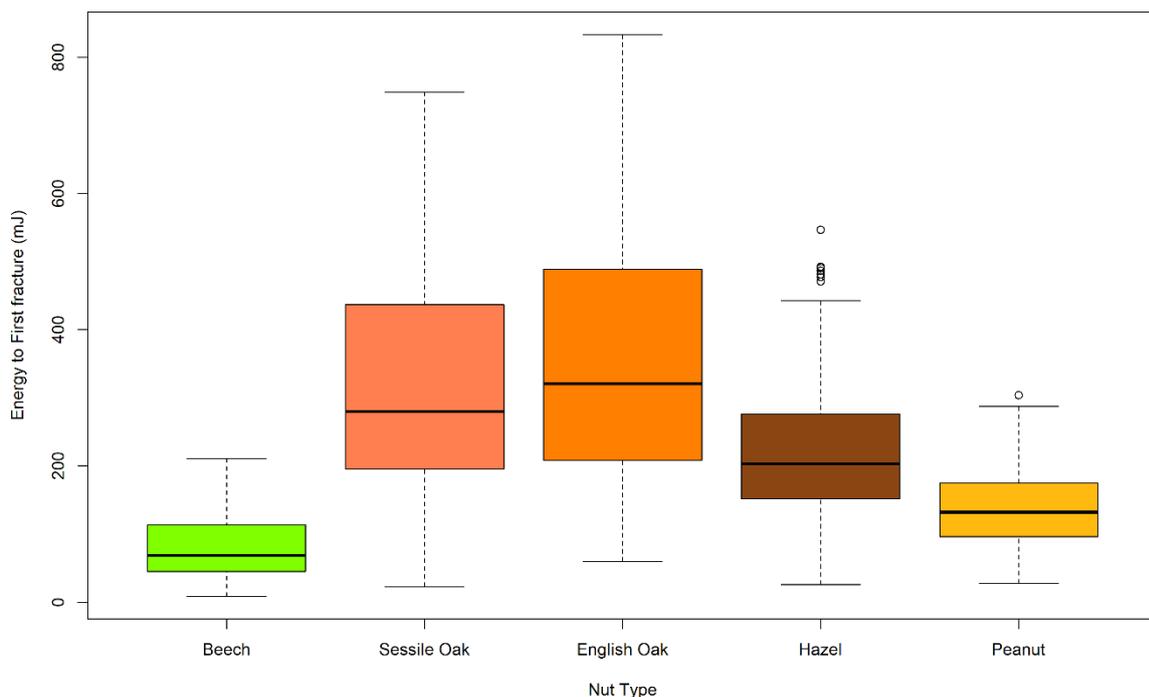


Figure 3.5. Boxplots showing energy (mJ) to initial fracture of a flat plate on non-cached whole nuts. Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.

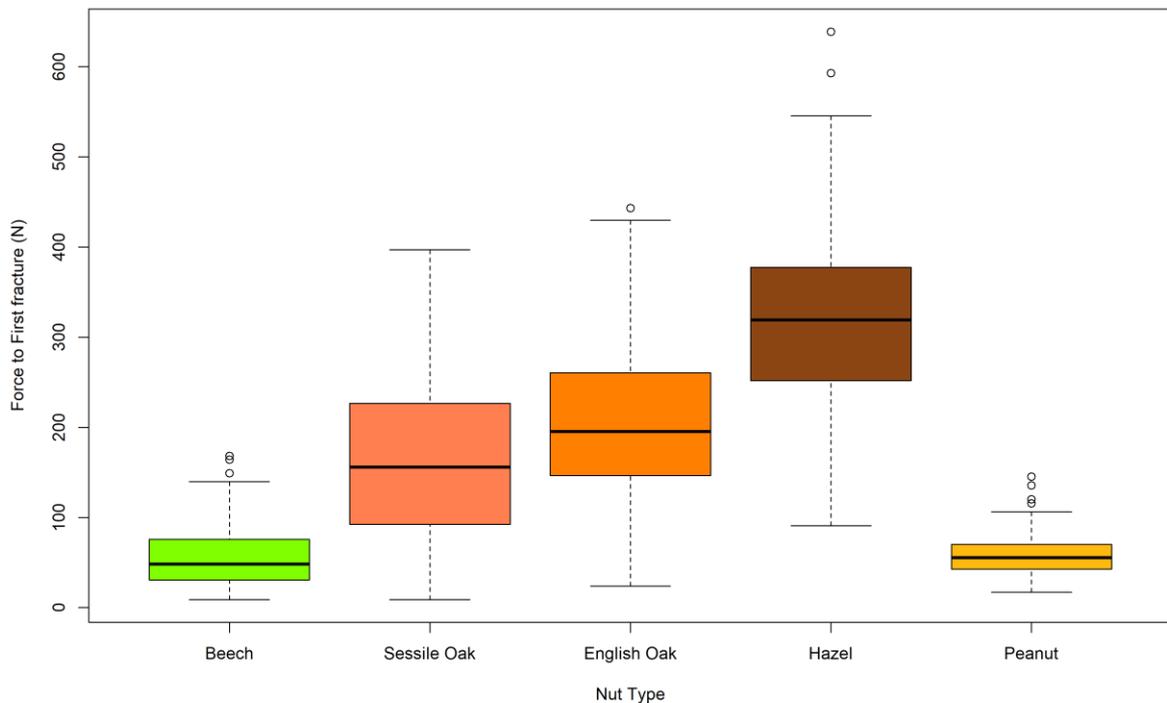


Figure 3.6. Boxplots showing force (N) to initial fracture of a flat plate on non-cached whole nuts. Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile

Analysis of cached samples with uncached samples (where all samples experiencing any form of caching were labelled as ‘cached’) were also performed (see Figures 3.7 and 3.8). Results of a two-way ANOVA on energy to initial fracture of all whole samples (cached and uncached), examining the effect of species and caching, showed significant differences between all species (ANOVA: $F = 185.4$, $p < 0.0001$) and between cached and uncached samples (ANOVA: $F = 16.0$, $p < 0.001$). Additionally, ANOVA showed that these variables interact with each other significantly ($F = 6.4$, $p < 0.001$). Further analysis, through pairwise Wilcoxon tests, determined that most sample conditions were significantly different from each other. Samples of cached and uncached peanuts and uncached bechnuts, had similar mean values and all interactions were insignificant ($p < 0.05$). Although affecting mean values overall, when all variations of caching were combined together in this way, there was limited effect, with bechnuts being the only species to have significant differences between cached and uncached samples (pairwise Wilcoxon: $p = 0.0001$).

For analysis of force to initial fracture, ANOVA detected significant differences between all species (ANOVA: $F = 509.1$, $p < 0.0001$) and between cached and uncached samples (ANOVA: $F = 21.9$, $p < 0.0001$). There was also a significant relationship detected between caching and species (ANOVA: $F = 4.065$, $p = 0.003$). An almost exact repeat of the energy to initial fracture results is seen with force to initial fracture. Pairwise Wilcoxon tests detected significant differences in mean values between all sample conditions except samples of cached and uncached peanuts and uncached hazelnuts ($p < 0.05$). Beechnuts are the only species to show significant differences between cached and uncached samples (pairwise Wilcoxon: $p = 0.0001$).

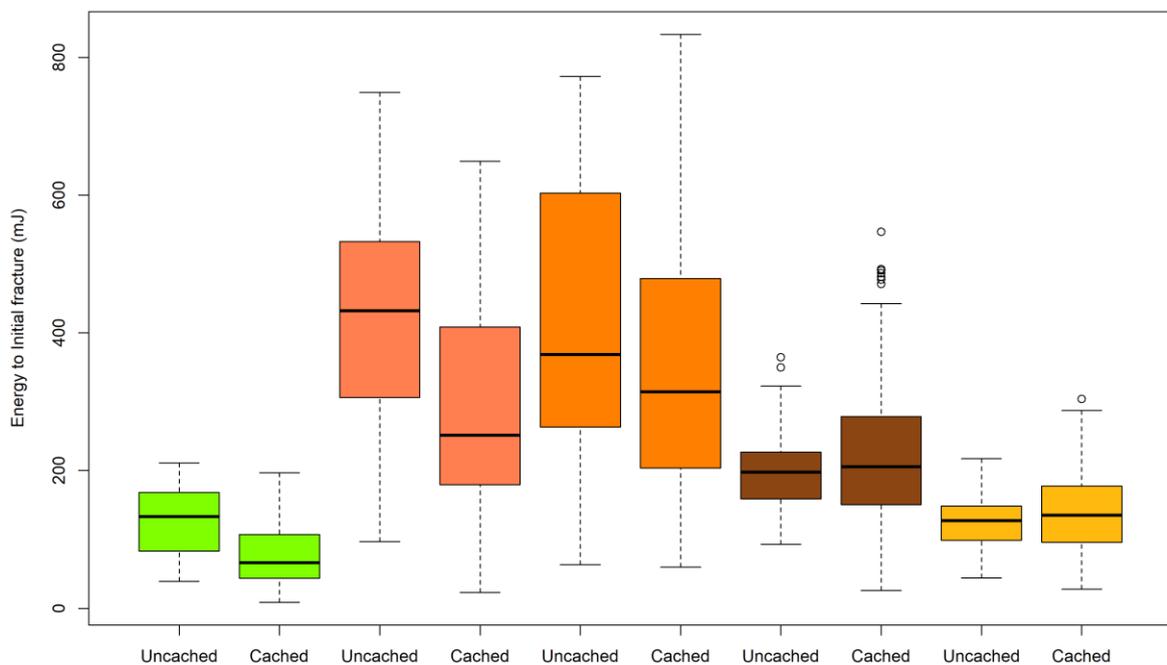


Figure 3.7. Boxplots showing energy (mJ) to initial fracture of a flat plate on non-cached whole nuts, with samples of both cached nuts and uncached nuts. Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.

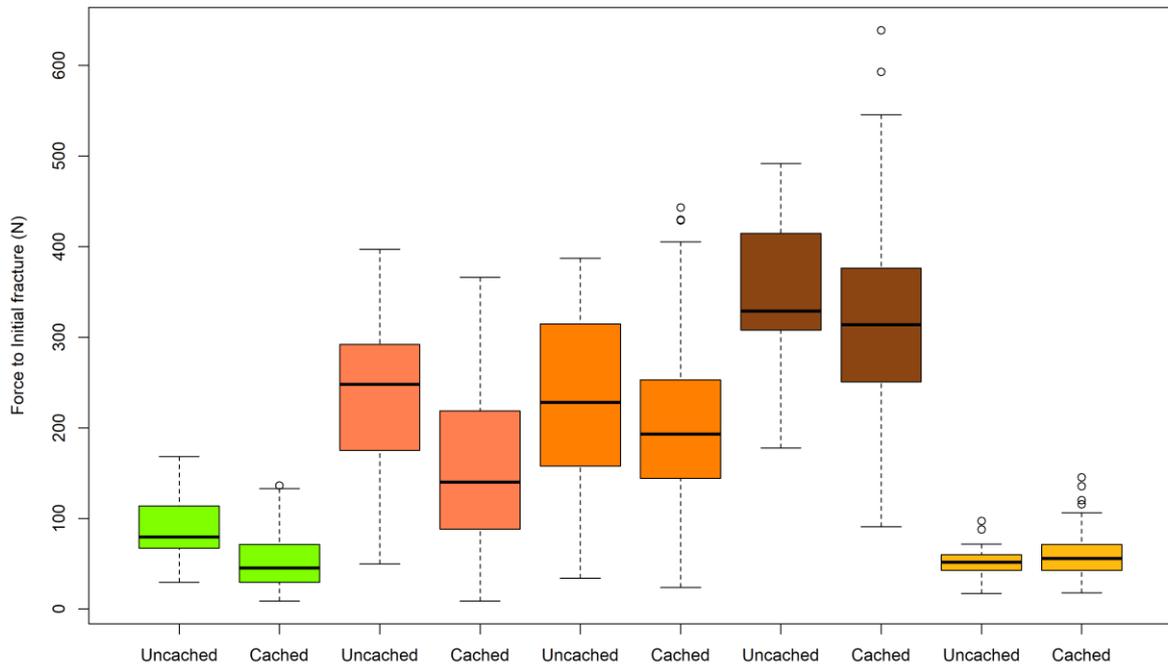


Figure 3.8. Boxplots showing force (N) to initial fracture of a flat plate on non-cached whole nuts, with samples of both cached nuts and uncached nuts. Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.

Table 3.2. Mean work-of-fracture/toughness seed shells or cone scales; energy to fracture (mJ) of whole nuts and force to fracture (N), of whole nuts of tree species regularly consumed by red squirrels *Sciurus vulgaris* in Britain.

	Work-of-fracture/toughness (mJ/mm)			Energy to Initial Fracture (mJ)		Force to Initial Fracture (N)	
	Uncached	Cached	Wild	Uncached	Cached	Uncached	Cached
Beechnut <i>Fagus sylvatica</i>	23.81	74.6	-	81.34	76.4	55.86	52.01
Sessile Oak <i>Quercus petraea</i>	28.01	21.97	-	310.59	289.65	164.82	152.5
English Oak <i>Quercus robur</i>	36.4	35.24	-	353.12	344.67	203.29	199.86
Hazelnut <i>Corylus avellana</i>	9.56	16.23	-	221.2	222.89	320.05	317.78
Peanut <i>Arachis hypogaea</i>	7.61	14.64	-	135.67	136.79	57.75	58.3
Sitka Spruce <i>Picea sitchensis</i>	14.48	25.6	-	-	-	-	-
Scots Pine <i>Pinus sylvestris</i>	10.86	10.77	8.13	-	-	-	-
Norway Spruce <i>Picea abies</i>	-	-	10.31	-	-	-	-

3.3.3 Caching Experiments

3.3.3.1 Beechnuts

Results of a three-way ANOVA on work-of-fracture of cached beechnut shells, examining the effect of caching temperature, caching substrate condition and time cached showed no significant effect of any variable. When including uncached samples in the analysis there was

a significant difference detected between uncached and cached samples, with uncached samples having significantly lower mean work-of-fracture than any cached samples (mean value of 23.82 mJ/mm while cached samples ranged between 50.65 - 98.94 mJ/mm) (see Figure 3.9).

Results of a three-way ANOVA on energy to fracture of whole cached beechnuts, examining the effect of caching temperature, caching substrate condition and time cached showed substrate condition to have a significant effect ($F = 3.1, p = 0.049$). No other variables had a significant effect. Similar results were obtained from a three-way ANOVA with the same variables on force to fracture with substrate condition having the only significant affect ($F = 4.7, p = 0.011$). Pairwise Wilcoxon tests analysing differences between uncached whole beechnuts and whole beechnuts cached in different substrate conditions showed damp sand to produce significantly lower energy values compared to no sand ($p = 0.041$) and dry sand ($p = 0.035$). This was also detected in force to fracture values compared to both no sand ($p = 0.0054$) and dry sand ($p = 0.015$). Generally, uncached whole beechnuts had higher mean values of energy and force to fracture than cached beechnuts (see Figures 3.9 and 3.10).

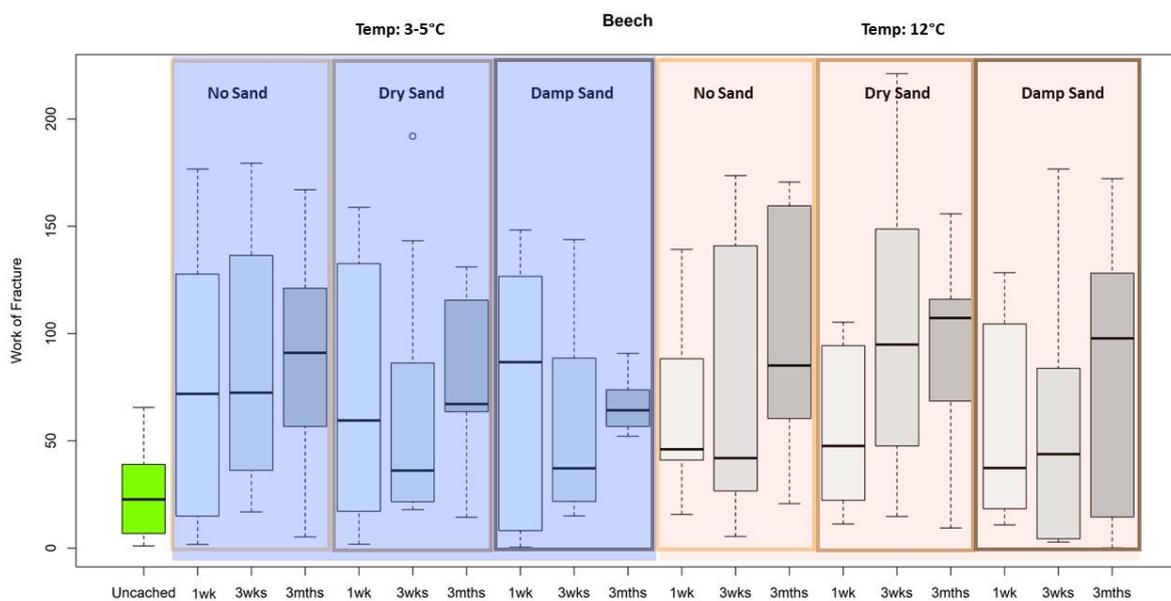


Figure 3.9. Boxplot showing work of fracture/toughness of segments of beechnut shells at different conditions for caching, including; time cached (in weeks/months), substrate condition and temperature stored (°C). Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.

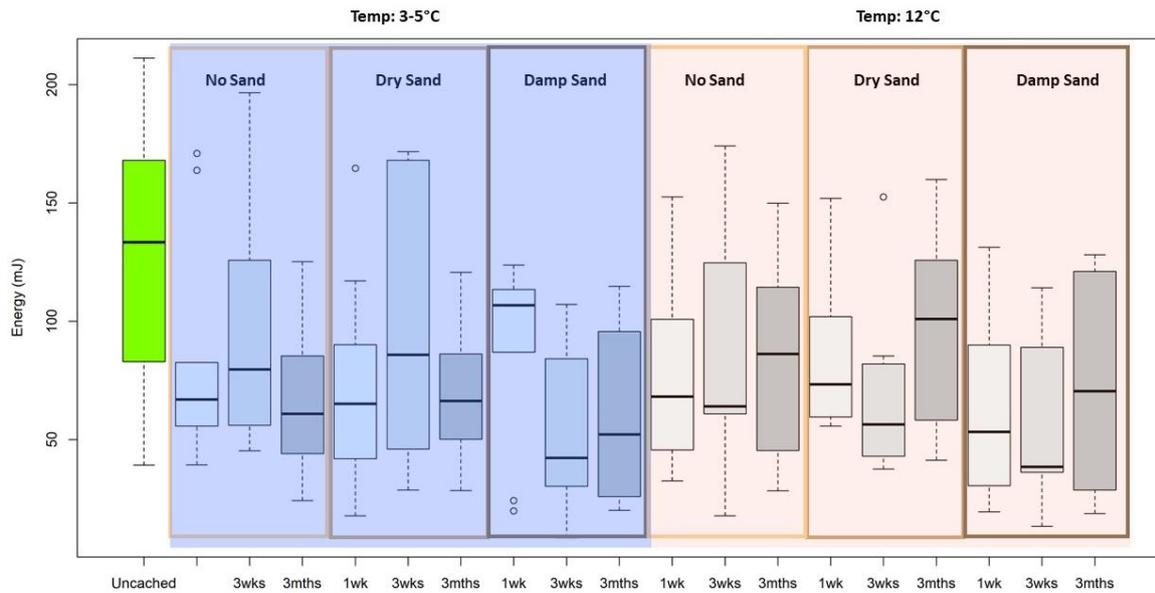


Figure 3.10. Boxplot showing energy (mJ) to initial fracture of a flat plate on whole bechnuts at different conditions for caching including time cached (in weeks/months), substrate condition and temperature stored (°C). Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.

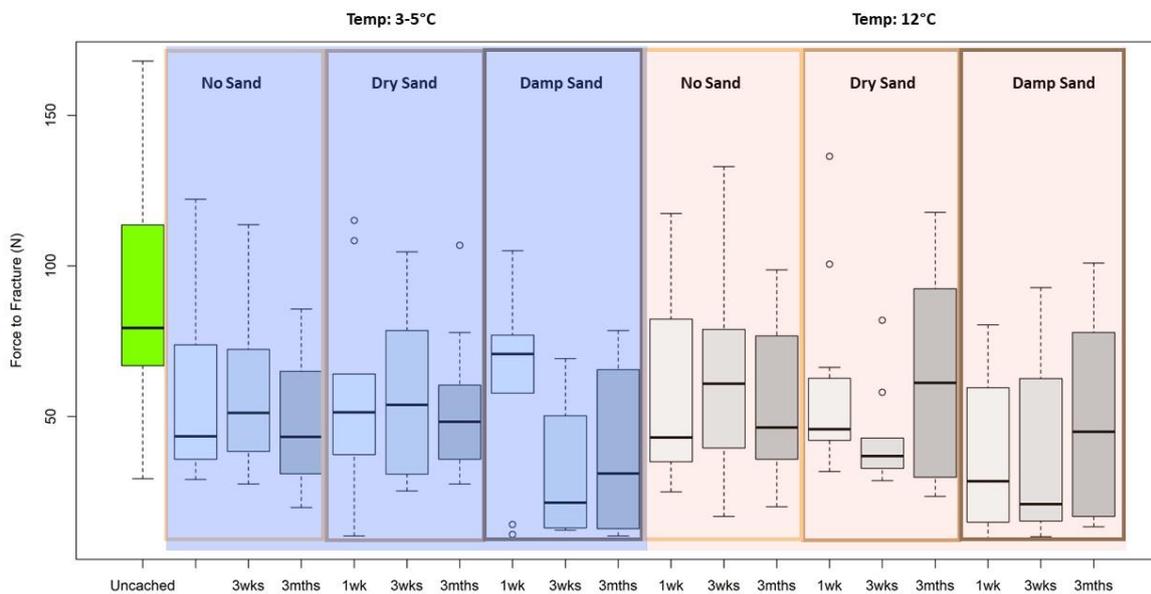


Figure 3.11. Boxplot showing energy (mJ) to initial fracture of a flat plate on whole bechnuts at different conditions for caching including time cached (in weeks/months), substrate condition and temperature stored (°C). Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.

3.3.3.2 – Sessile Oak

Sessile oak acorns, were the only species, other than Scots pine or Sitka spruce, that showed a significant difference in work-of-fracture values between samples cut parallel or perpendicular to the axis of growth (Kruskal-Wallis chi-squared = 52.492, $df = 1$, p -value < 0.001), with samples cut parallel being significantly lower than those cut perpendicular (mean work-of-fracture of 12.97 compared to 32.29).

When including all samples, results of a three-way ANOVA on work-of-fracture on cached Sessile Oak acorn shells, showed both substrate condition ($F = 3.4$, $p = 0.037$) and time cached ($F = 6.4$, $p < 0.003$) to have significant effects (see Figure 3.12). When analysed with a smaller subset, of samples only cut parallel to the growth axis, the results showed both temperature ($F = 7.9$, $p < 0.007$) and time cached ($F = 8.8$, $p < 0.001$) to have significant effects (see Figure 3.13). For both analyses, samples cached for 3 weeks generally required lower work-of-fracture than other times cached (including uncached samples) and samples cached in damp sand generally required lower work-of-fracture than other substrate conditions. The difference in mean work-of-fracture between cached and uncached samples was wider in analyses including only samples cut parallel to the axis of growth.

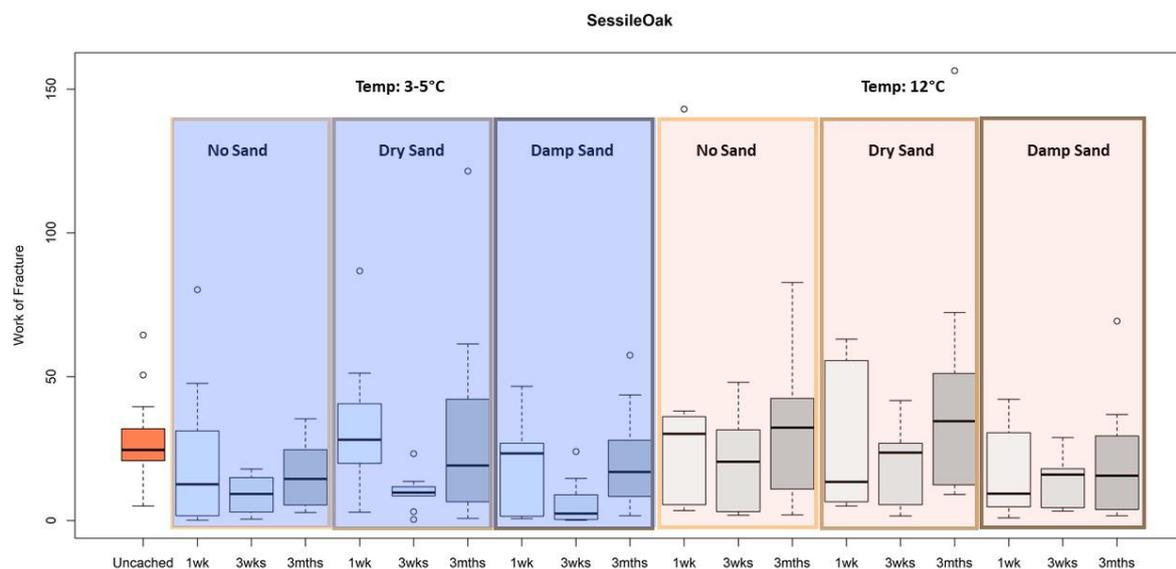


Figure 3.12. Boxplot showing work of fracture/toughness of segments of sessile oak acorn (cut with and against the grain) at different conditions for caching, including; time cached (in weeks/months), substrate condition and temperature stored (°C). Black line represents median, box represents interquartile range from the box.

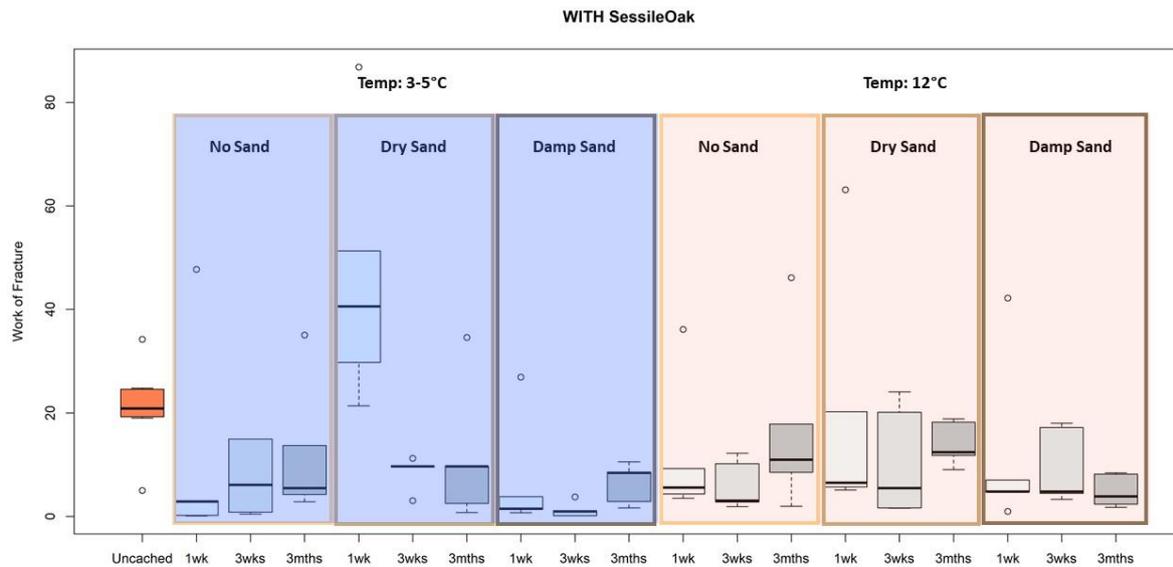


Figure 3.13. Boxplot showing work of fracture/toughness of segments of sessile oak acorn (cut with the grain only) at different conditions for caching, including; time cached (in weeks/months), substrate condition and temperature stored (°C). Black line represents median, box represents interquartile range from the box.

The majority of sessile oak acorns were infected with mould by 3 months, which had largely disintegrated the shell of the acorns. These samples were therefore removed from the analysis of energy to fracture and force to fracture entirely.

Results of a three-way ANOVA on energy to fracture on whole cached Sessile Oak acorns, showed significant effect of all three variables of caching temperature ($F = 10.0$, $p = 0.002$), caching substrate condition ($F = 5.6$, $p < 0.005$) and time cached ($F = 3.9$, $p < 0.03$) (see Figure 3.14). Additionally, temperature interacted to have a significant affect with both moisture ($F = 3.9$, $p < 0.03$) and time cached ($F = 5.2$, $p < 0.007$). Generally, samples cached at 3-5°C required higher energy to fracture than samples cached at 12°C. Samples cached for 1 week in colder conditions required higher work-of-fracture than samples cached for more than 1 week, while the reverse is true of those samples cached in warmer conditions. Generally, samples cached in no sand or dry sand required lower work-of-fracture than uncached samples for those cached in damp sand, a difference that was more pronounced in warmer conditions. Almost all samples, required significantly less energy to fracture than uncached samples.

Results of a three-way ANOVA on force to fracture with the same variables showed a significant effect of substrate condition ($F = 4.1, p < 0.02$) and time cached ($F = 7.9, p < 0.0007$) (see Figure 3.15). Samples cached in damp sand were generally higher and so were samples cached for one week; however these results would likely be different if 3 month cached samples had not been removed from the dataset. Again, most cached samples required a lower force to fracture than uncached samples.

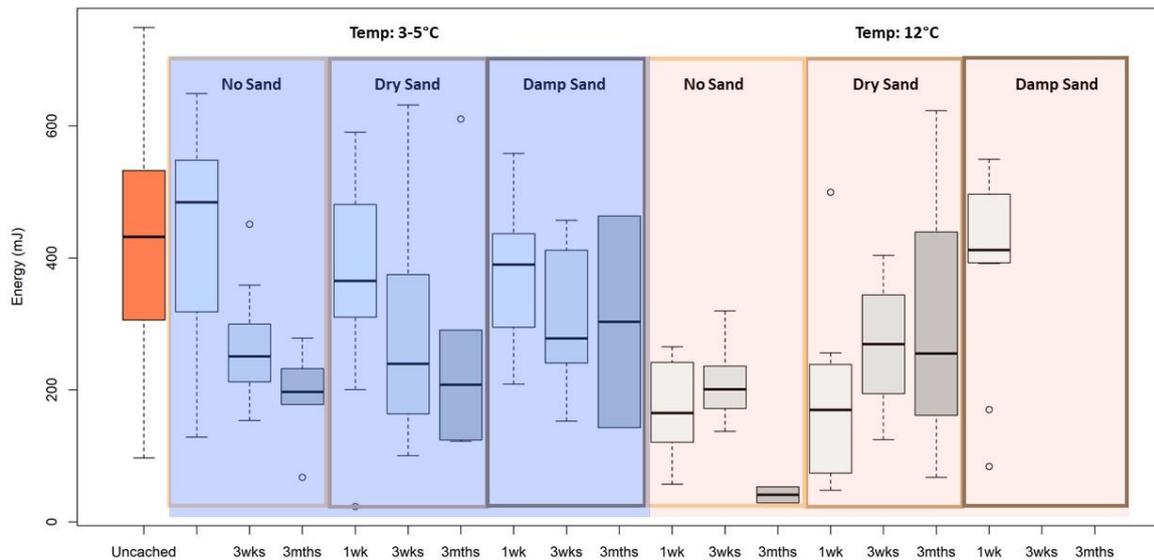


Figure 3.14. Boxplot showing energy (mJ) to initial fracture of a flat plate on whole sessile oak acorns at different conditions for caching including time cached (in weeks/months), substrate condition and temperature stored (°C). Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.

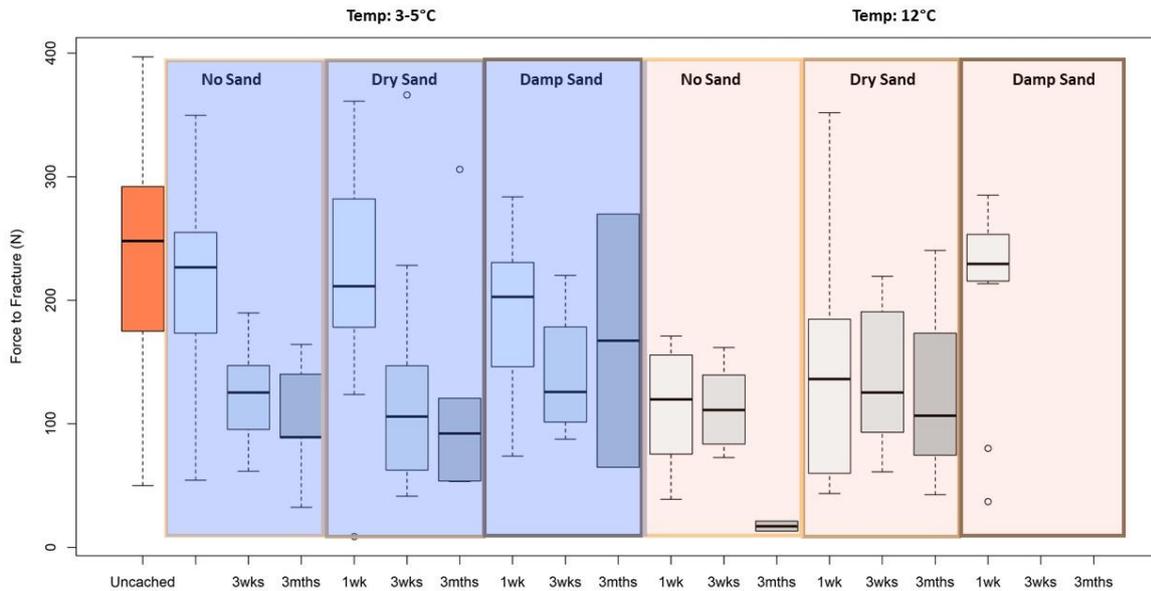


Figure 3.15. Boxplot showing force (N) to initial fracture of a flat plate on whole beech nuts at different conditions for caching including time cached (in weeks/months), substrate condition and temperature stored (°C). Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.

3.3.3.3 English Oak

Results of a three-way ANOVA on work-of-fracture on cached English Oak acorns shell, examining the effect of caching temperature, caching substrate condition and time cached showed a significant effect of time cached ($F = 4.3$, $p = 0.015$) (see Figure 3.16). Pairwise Wilcoxon tests on uncached and cached samples of English oak acorn shells determined that samples cached for 1 week required significantly lower work-of-fracture compared to other samples ($p = 0.006$). Additionally, three-way ANOVA demonstrated that substrate condition and temperature interact to have a significant effect ($F = 3.9$, $p = 0.023$).

Most of the samples stored in damp sand at 12°C for 3 months had sprouted, so this group was removed from the analysis of energy and force to fracture. Results of a three-way ANOVA on energy to fracture on whole cached acorns, showed significant effect of substrate condition ($F = 30.8$, $p < 0.002$) and time cached ($F = 130.4$, $p < 0.002$) (see Figure 3.17). Additionally, substrate conditions together with temperature effected energy to fracture ($F = 5.1$, $p < 0.008$) and so did substrate conditions together with time cached ($F = 5.2$, $p <$

0.0006). Samples cached for 1 week required considerably lower energy to fracture (mean 184.4 mJ) than other samples including uncached samples (mean 422.79 mJ) whilst samples cached for 3 weeks required the highest amount of energy (mean 479.98 mJ). Samples cached in damp sand showed a more extreme diversity with the gap in energy required between samples cached for 1 week and those cached for 3 weeks to be considerably wider than in other substrate conditions.

Three-way ANOVA on force to fracture showed significant effects of all three variables (temperature: $F = 5.5$ $p = 0.0204$; substrate condition: $F = 60.2$ $p < 0.001$; and time cached $F = 3.9$ $p = 0.022$) (see Figure 3.18). However none of these variables interacted with each other significantly. Generally samples cached for 1 week required lower force to fracture compared to other caching times. Samples cached in damp sand generally required higher force to fracture than other substrates, as did samples cached at 12°C compared to those cached at 3-5°C.

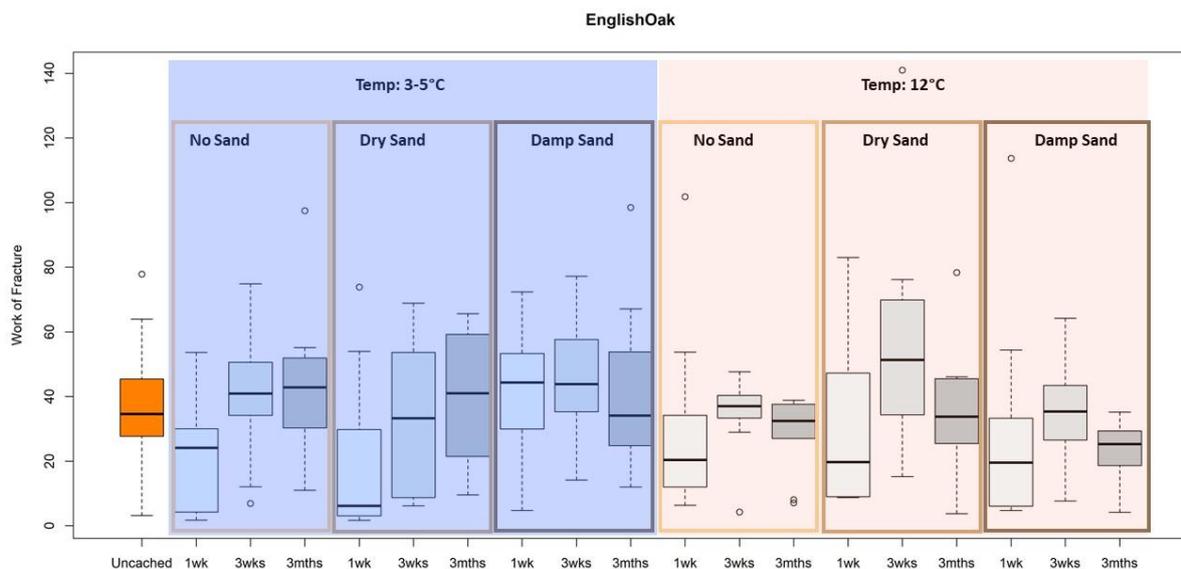


Figure 3.16. Boxplot showing work of fracture/toughness of segments of English oak acorn shells at different conditions for caching, including; time cached (in weeks/months), substrate condition and temperature stored (°C). Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.

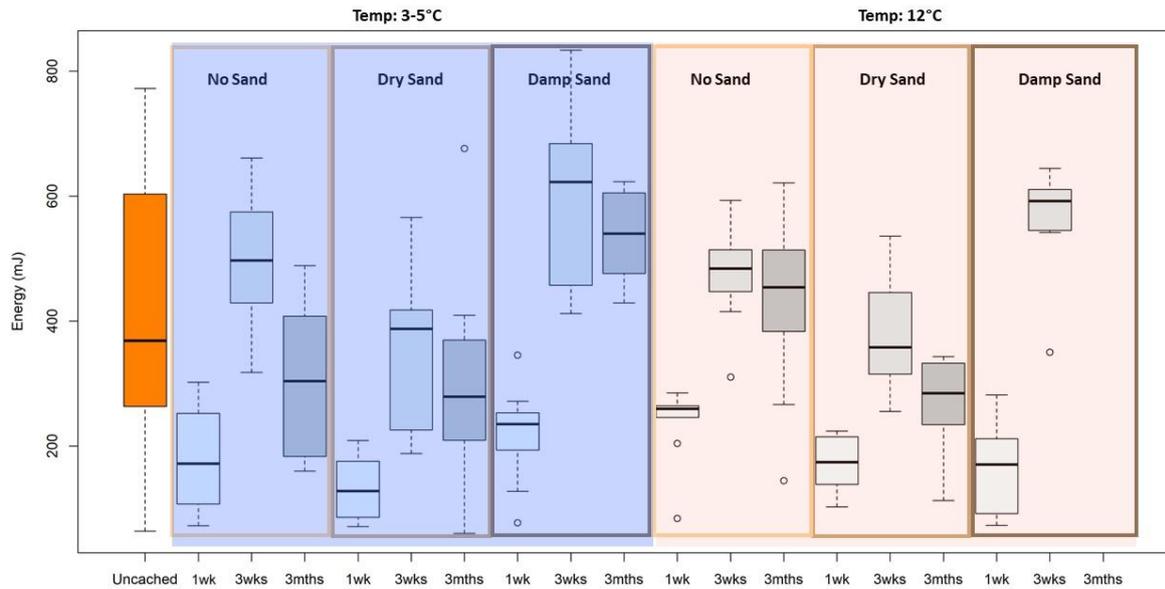


Figure 3.17. Boxplot showing energy (mJ) to initial fracture of a flat plate on whole English oak acorns at different conditions for caching including time cached (in weeks/months), substrate condition and temperature stored (°C). Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.

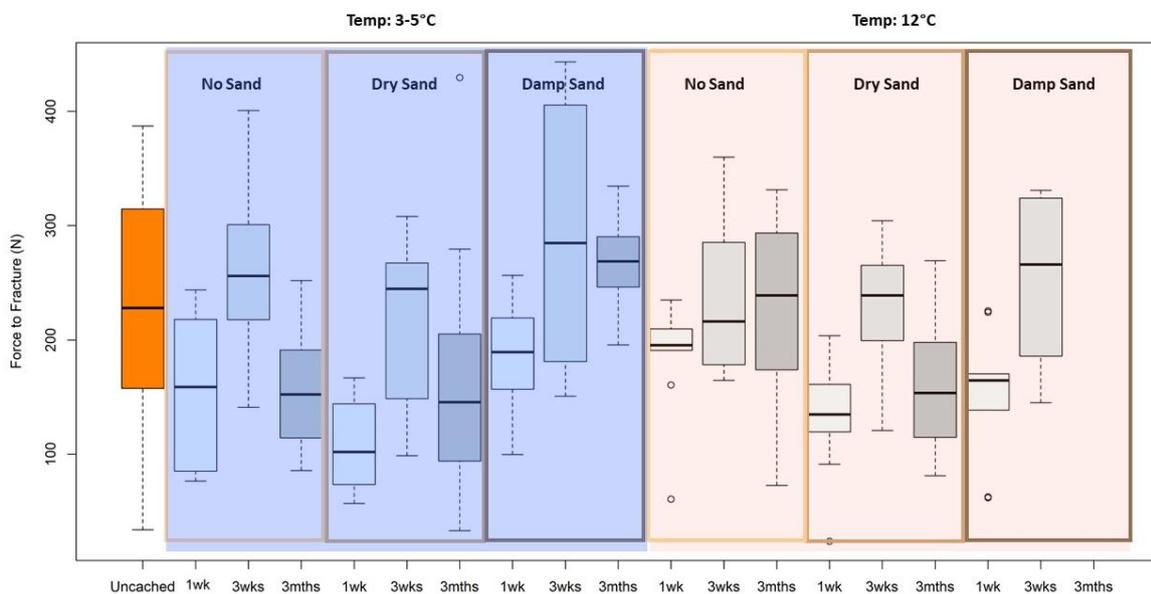


Figure 3.18. Boxplot showing force (N) to initial fracture of a flat plate on whole English oak acorns at different conditions for caching including time cached (in weeks/months), substrate condition and temperature stored (°C). Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.

3.3.3.4 Hazelnuts

Results of a three-way ANOVA on work-of-fracture of cached hazelnut shells, examining the effect of caching temperature, caching substrate condition and time cached, showed that both substrate condition ($F = 6.9$, $p < 0.002$) and time cached ($F = 40.9$, $p < 0.001$) had an effect (see Figure 3.19). Pairwise Wilcoxon tests detected significant differences in work-of-fracture between caching times, except uncached samples and those cached for 3 months, which were virtually the same. Uncached specimens had significantly lower work-of-fracture values. Pairwise Wilcoxon tests of substrate conditions detected that uncached samples produced significantly lower work-of-fracture values than all cached samples and damp sand produced significantly higher work-of-fracture values than other samples.

A three-way ANOVA on energy to fracture and force to fracture of whole cached hazelnut shells, showed a significant effect of both substrate condition ($F = 9.3$, $p < 0.001$) and time cached ($F = 10.1$, $p < 0.001$). Additionally, these variables interacted in a way that also had an effect ($F = 8.7$, $p < 0.001$). This was evident in that samples cached in damp sand had significantly lower energy to fracture than other samples, including uncached samples and samples cached for 3 months generally required lower energy to fracture. The interaction between these variables meant samples cached for 3 months in damp sand required the lowest energy of all samples (see Figures 3.20 and 3.21).

For force to fracture, all three variables of temperature ($F = 5.5$, $p = 0.02$), substrate condition ($F = 60.2$, $p < 0.001$) and time cached ($F = 3.9$, $p < 0.03$) had an effect but these did not interact with each other significantly. For temperature and time cached the differences were significant, but minimal. Samples cached for 3 months generally required less force to fracture, but not a considerable difference to other caching times. Samples cached at 12°C generally required lower force to fracture than those cached at $3\text{-}5^{\circ}\text{C}$. The largest differences were between substrate conditions, in which samples cached in damp sand required considerably lower force to fracture than other samples, including uncached samples.

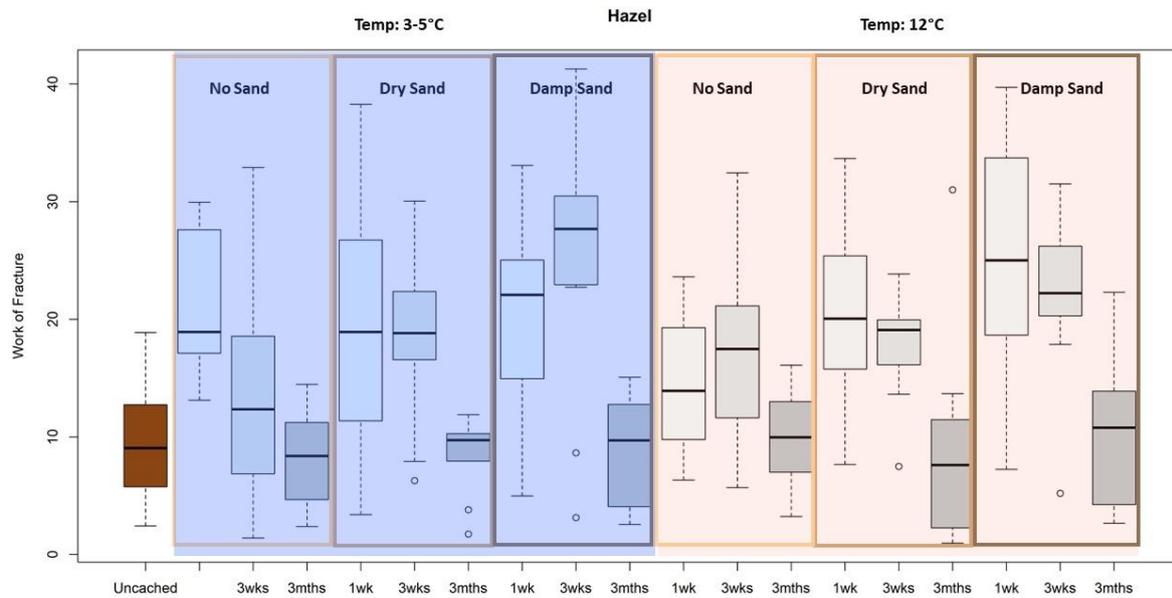


Figure 3.19. Boxplot showing work of fracture/toughness of segments of hazelnut shells at different conditions for caching, including; time cached (in weeks/months), substrate condition and temperature stored (°C). Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.

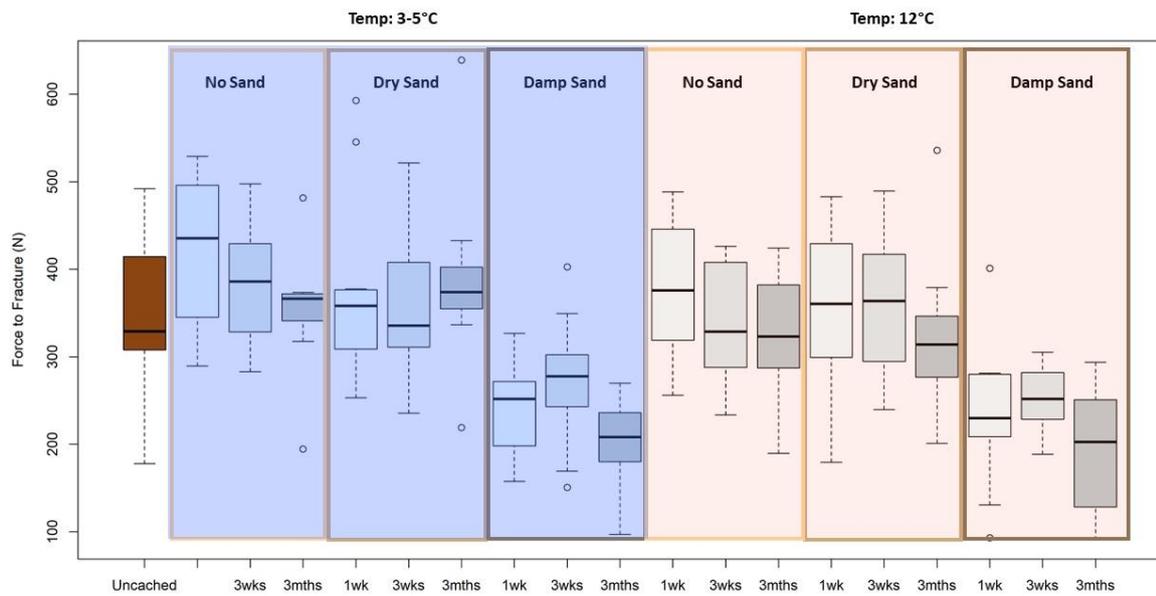


Figure 3.20. Boxplot showing energy (mJ) to initial fracture of a flat plate on whole hazelnut at different conditions for caching including time cached (in weeks/months), substrate condition and temperature stored (°C). Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.

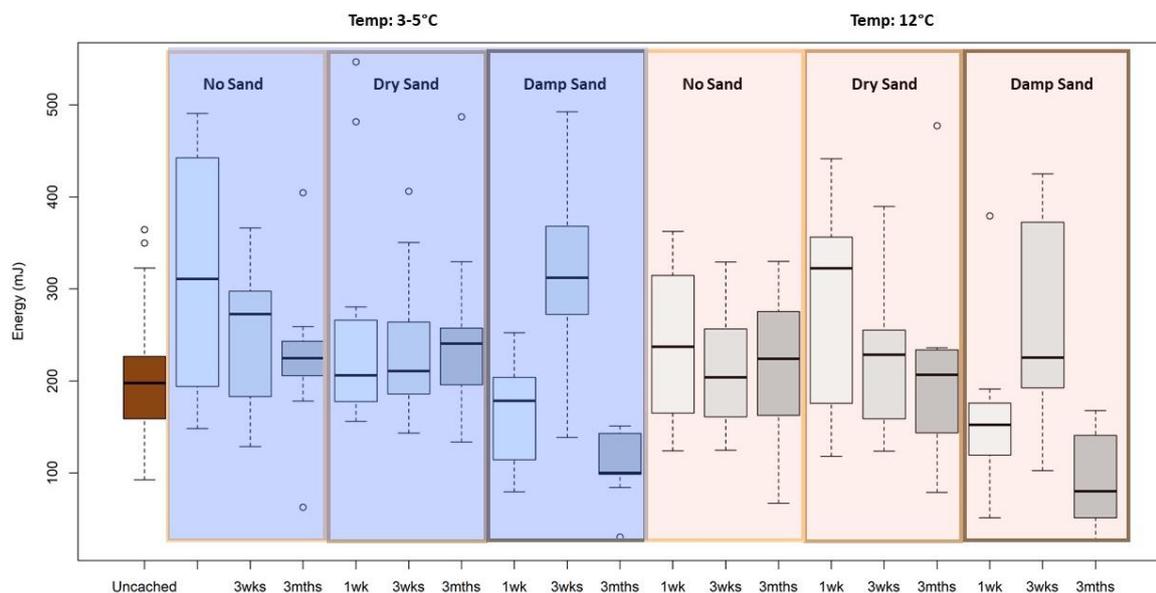


Figure 3.21. Boxplot showing force (N) to initial fracture of a flat plate on whole hazelnut at different conditions for caching including time cached (in weeks/months), substrate condition and temperature stored (°C). Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.

3.3.3.5 Peanuts

Results of a three-way ANOVA on work-of-fracture of cached peanut shells, examining the effect of caching temperature, caching substrate condition and time cached showed only time cached to have an effect ($F = 23.4$, $p < 0.001$). Pairwise Wilcoxon tests comparing work-of-fracture across caching times of both uncached and cached samples were performed, and showed no significant differences between uncached samples and samples cached for 3 months. Both these samples had significantly lower work-of-fracture values than samples cached for 1 week or 3 weeks (See Figure 3.22).

Three-way ANOVA on energy to fracture of cached whole peanut shells, only detected a significant effect of substrate condition ($F = 12.5$, $p < 0.001$) but this also interacted with time cached to have an effect ($F = 2.5$, $p < 0.05$). A similar pattern was seen in analysis of force to fracture with substrate condition having a significant effect ($F = 12.0$, $p < 0.001$) and interacting with time cached to also have a significant effect ($F = 2.8$, $p = 0.026$). Samples cached in damp sand generally required lower energy/force to fracture, and samples cached in damp sand for more than 1 week required the lowest energy/force to fracture of all conditions (see Figures 3.23 and 3.24).

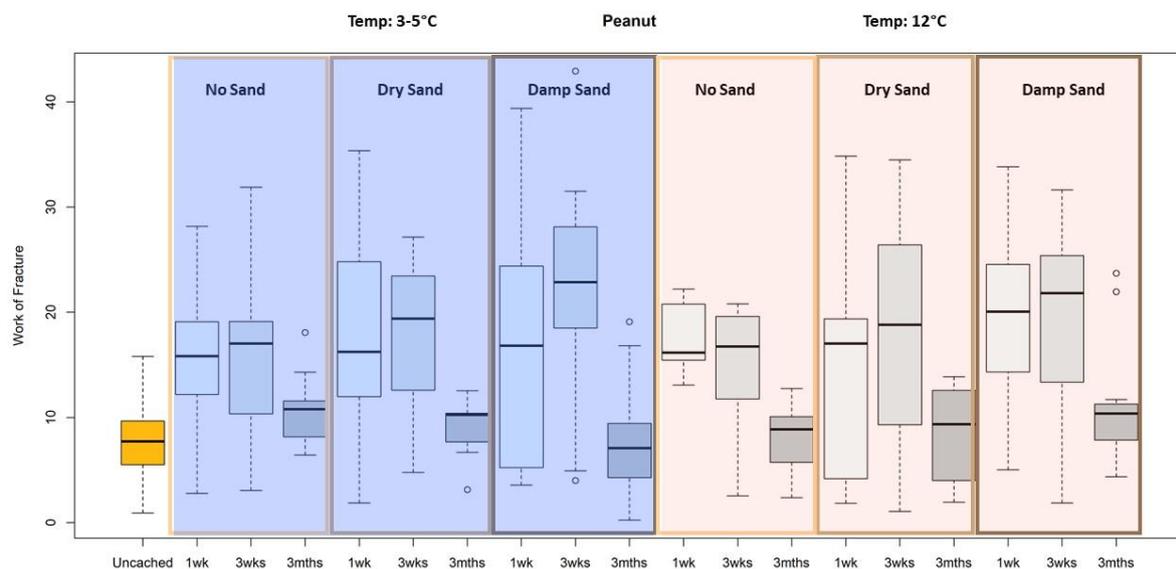


Figure 3.22. Boxplot showing work of fracture/toughness of segments of peanut shells at different conditions for caching, including; time cached (in weeks/months), substrate condition and temperature stored (°C). Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.

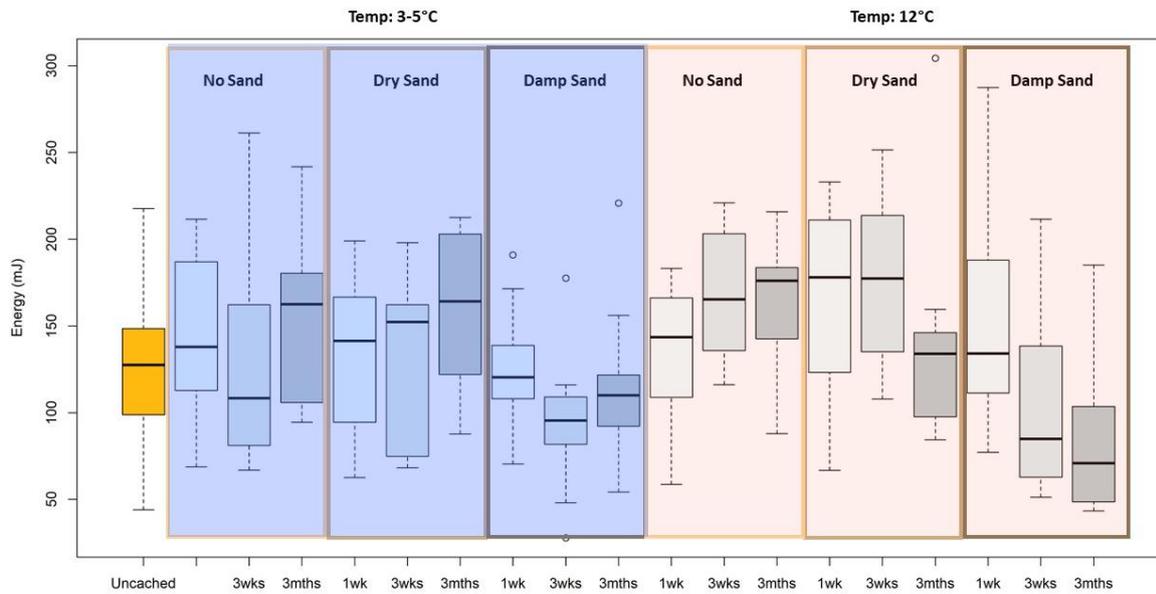


Figure 3.23. Boxplot showing energy (mJ) to initial fracture of a flat plate on whole peanuts at different conditions for caching including time cached (in weeks/months), substrate condition and temperature stored (°C). Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.

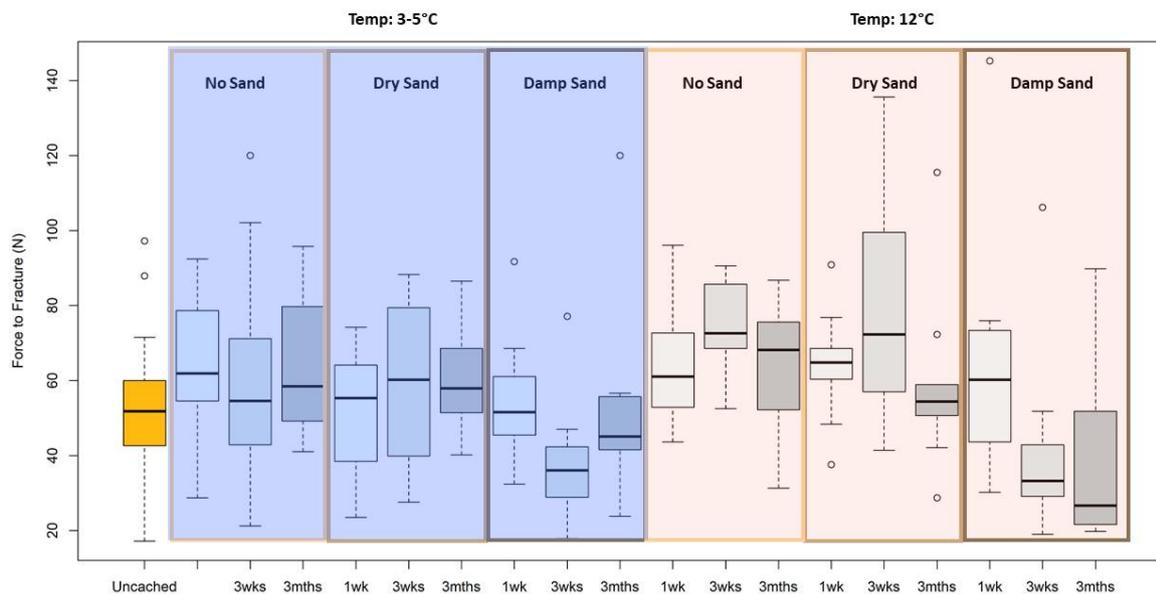


Figure 3.24. Boxplot showing force (N) to initial fracture of a flat plate on whole peanuts at different conditions for caching including time cached (in weeks/months), substrate condition and temperature stored (°C). Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.

3.3.3.6 Sitka Spruce

Results of a three-way ANOVA on work-of-fracture of cached Sitka spruce pine cone scales, examining the effect of caching temperature, caching substrate condition and time cached showed both substrate condition ($F = 21.7$, $p < 0.001$) and time cached ($F = 30.8$, $p < 0.001$) to have a significant effect (see Figure 3.25). Both variables also interacted significantly ($F = 5.8$, $p < 0.001$). Samples cached in no sand or dry sand had relatively high values, higher than uncached samples, when cached for 1 or 3 weeks. Samples cached in damp sand however, had consistently low values, across all caching times. Pairwise Wilcoxon tests performed on all samples, including uncached samples, demonstrated that samples cached for 3 weeks had significantly higher mean values than other samples ($p < 0.05$ for all interactions).

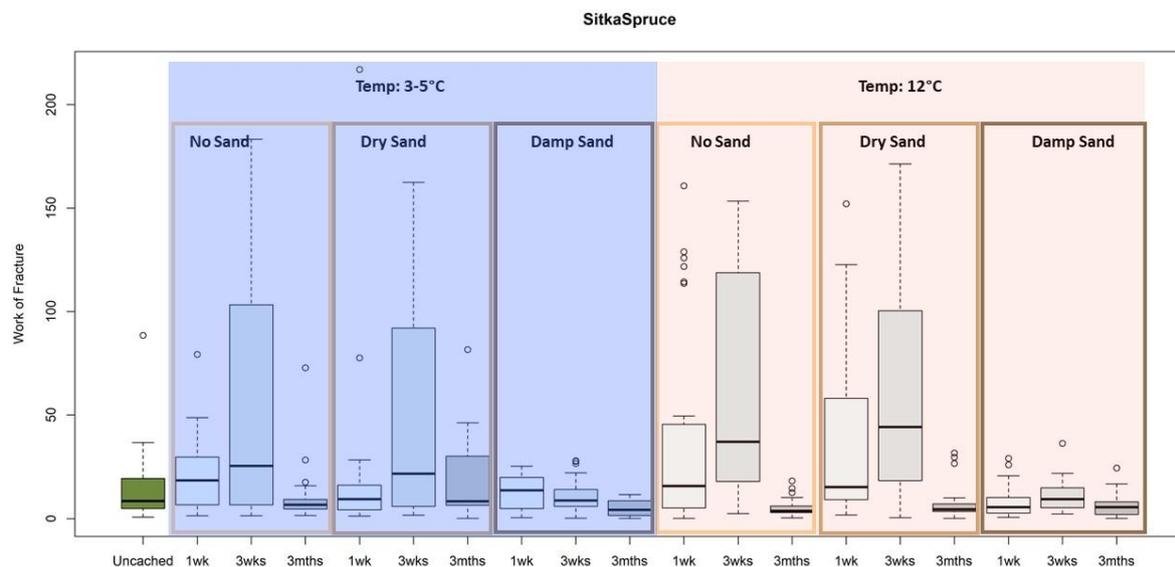


Figure 3.25. Boxplot showing work of fracture/toughness of segments of sitka spruce cone scales at different conditions for caching, including; time cached (in weeks/months), substrate condition and temperature stored (°C). Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.

3.3.3.7 Scots Pine

Results of a three-way ANOVA on work-of-fracture of cached Scots pine cone scales, examining the effect of caching temperature, caching substrate condition and time cached showed both substrate condition ($F = 7.8$, $p < 0.001$) and time cached to have an effect ($F = 5.5$, $p < 0.005$) (see Figure 3.26). Pairwise Wilcoxon tests on all samples, cached and

uncached, showed that samples cached in damp sand had significantly lower work-of-fracture values than all other substrate conditions. ($p < 0.01$ for all interactions). Samples cached for 3 months generally had lower mean work-of-fracture than other samples, although not significantly different from uncached samples.

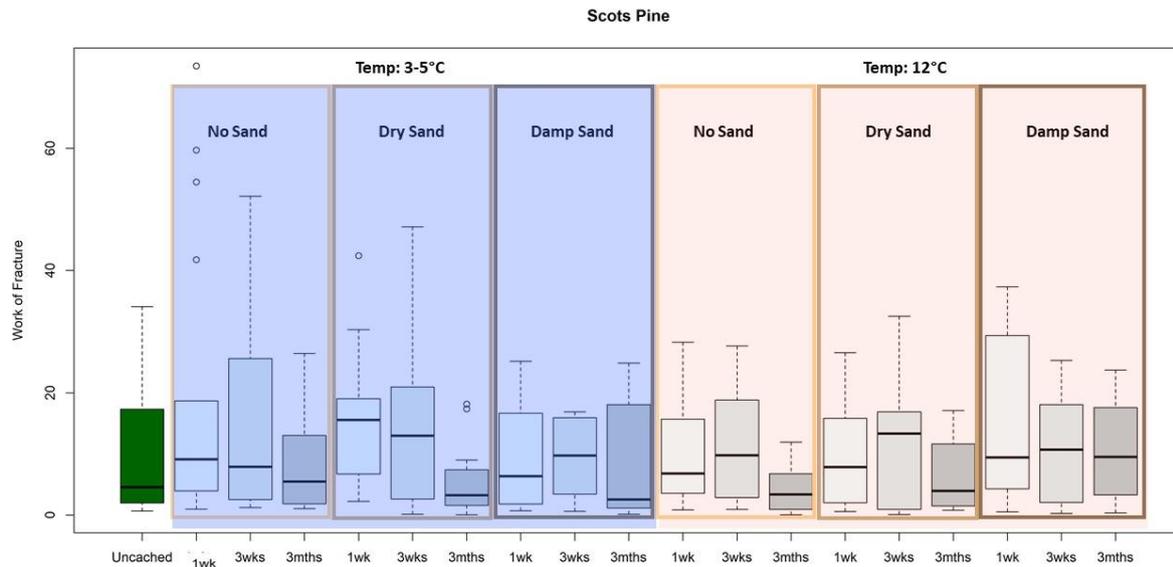


Figure 3.26. Boxplot showing work of fracture/toughness of segments of scots pine cone scales at different conditions for caching, including; time cached (in weeks/months), substrate condition and temperature stored ($^{\circ}\text{C}$). Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.

Table 3.3. Results of three-way ANOVAs on work-of-fracture of cached shells or scales of food items commonly consumed by British populations of red squirrels, examining the effect of caching temperature, caching substrate condition and time cached, and interactions between these variables.

	Temp	Substrate Condition	Time Cached	Temp * Moisture	Temp * Time	Substrate Condition * Time	Temp * Substrate Condition * Time
Beechnut <i>Fagus sylvatica</i>	F = 0.3 p = 0.57	F = 1.5 p = 0.231	F = 1.9 p = 0.14	F = 0.6 p = 0.53	F = 2.3 p = 0.107	F = 0.2 p = 0.923	F = 0.4 p = 0.777
Sessile Oak <i>Quercus petraea</i>	F = 3.6 p = 0.06	F = 3.4 p = 0.037 *	F = 6.4 p = 0.002 **	F = 0.9 p = 0.422	F = 0.7 p = 0.522	F = 0.7 p = 0.613	F = 0.3 p = 0.852
Sessile Oak <i>Quercus petraea</i> (parallel to growth axis)	F = 7.9 p < 0.007 **	F = 1.6 p = 0.214	F = 8.8 p < 0.001 ***	F = 2.5 p = 0.0928	F = 0.3 p = 0.713	F = 2.7 p = 0.0348 *	F = 0.1 p = 0.994
English Oak <i>Quercus robur</i>	F = 0.6 p = 0.46	F = 0.3 p = 0.733	F = 4.3 p = 0.015* *	F = 3.9 p = 0.023 *	F = 2.3 p = 0.1	F = 0.9 p = 0.411	F = 0.5 p = 0.738
Hazelnut <i>Corylus avellana</i>	F = 0.1 p = 0.75	F = 6.8 p < 0.001 **	F = 40.9 p < 0.001 ***	F = 0.1 p = 0.893	F = 0.3 p = 0.713	F = 1.5 p = 0.219	F = 2.0 p = 0.093
Peanut <i>Arachis hypogaea</i>	F = 0.2 p = 0.64	F = 1.5 p = 0.235	F = 23.5 p < 0.001 ***	F = 0.3 p = 0.71	F = 0.3 p = 0.739	F = 0.6 p = 0.669	F = 0.7 p = 0.603

Sitka Spruce <i>Picea sitchensis</i>	F = 1.6 p = 0.21	F = 21.7 p < 0.001 ***	F = 30.8 p < 0.001 ***	F = 0.5 p = 0.59	F = 1.8 p = 0.174	F = 5.8 p = 0.001 ***	F = 0.8 p = 0.523
Scots Pine <i>Pinus sylvestris</i>	F = 0.5 p = 0.46	F = 7.8 p < 0.001 ***	F = 5.5 p = 0.005 **	F = 0.2 p = 0.84	F = 1.4 p = 0.232	F = 0.9 p = 0.464	F = 0.087 p = 0.917

Table 3.4. Results of three-way ANOVAs on energy to fracture (mJ) of whole cached food items commonly consumed by British populations of red squirrels, examining the effect of caching temperature, caching substrate condition and time cached, and interactions between these variables.

	Temp	Substrate Condition	Time Cached	Temp * Moisture	Temp * Time	Substrate Condition * Time	Temp * Substrate Condition * Time
Beechnut <i>Fagus sylvatica</i>	F = 0.1 p = 0.961	F = 3.1 p < 0.049 *	F = 0.1 p = 0.881	F = 0.04 p = 0.958	F = 2.8 p = 0.063	F = 0.8 p = 0.478	F = 0.9 p = 0.457
Sessile Oak <i>Quercus petraea</i>	F = 10.0 p < 0.003 **	F = 5.6 p < 0.005 **	F = 3.9 p < 0.022 *	F = 3.9 p < 0.023 *	F = 5.2 p < 0.007 **	F = 1.5 p = 0.196	F = 0.5 p = 0.617
English Oak <i>Quercus robur</i>	F = 1.660 p = 0.199	F = 30.8 p < 0.001 ***	F = 130.4 p < 0.001 ***	F = 5.1 p = 0.007 **	F = 0.5 p = 0.621	F = 5.2 p < 0.001 ***	F = 1.8 p = 0.143
Hazelnut <i>Corylus avellana</i>	F = 3.5 p = 0.063	F = 9.3 p < 0.001 ***	F = 10.1 p < 0.001 ***	F = 0.5 p = 0.619	F = 0.1 p = 0.897	F = 8.7 p < 0.001 ***	F = 0.8 p = 0.5

Peanut <i>Arachis hypogaea</i>	F = 3.0 p = 0.083	F = 12.5 p < 0.001 ***	F = 0.4 p = 0.676	F = 0.5 p = 0.618	F = 2.5 p = 0.081	F = 2.5 p = 0.048 *	F = 1.4 p = 0.22
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Table 4.5. Results of three-way ANOVAs on force to fracture (N) of whole cached food items commonly consumed by British populations of red squirrels, examining the effect of caching temperature, caching substrate condition and time cached, and interactions between these variables.

	Temp	Substrate Conditio n	Time Cached	Temp * Moisture	Temp * Time	Substrate Conditio n * Time	Temp * Substrate Conditio n * Time
Beechnut <i>Fagus sylvatica</i>	F = 0.01 p = 0.949	F = 4.6 p = 0.01 *	F = 0.8 p = 0.433	F = 0.3 p = 0.759	F = 1.1 p = 0.339	F = 1.0 p = 0.406	F = 0.9 p = 0.48
Sessile Oak <i>Quercus petraea</i>	F = 1.9 p = 0.17	F = 4.1 p = 0.019 *	F = 7.9 p < 0.001 ***	F = 1.8 p = 0.17	F = 2.1 p = 0.132	F = 0.5 p = 0.718	F = 0.3 p = 0.715
English Oak <i>Quercus robur</i>	F = 0.4 p = 0.54	F = 10.2 p < 0.001 ***	F = 26.3 p < 0.001 ***	F = 3.9 p = 0.021 *	F = 0.6 p = 0.569	F = 0.7 p = 0.561	F = 1.2 p = 0.28
Hazelnut <i>Corylus avellana</i>	F = 5.5 p < 0.03 *	F = 60.2 p < 0.001 ***	F = 3.9 p = 0.022 *	F = 0.4 p = 0.666	F = 0.2 p = 0.838	F = 1.36 p = 0.247	F = 0.5 p = 0.73
Peanut <i>Arachis hypogaea</i>	F = 2.14 p = 0.145	F = 11.9 p < 0.001 ***	F = 0.5 p = 0.58	F = 0.8 p = 0.44	F = 2.4 p = 0.093	F = 2.8 p = 0.026 *	F = 0.5 p = 0.701

3.3.4 *Wild Samples*

Analysis was performed on Scots pine samples from three locations; the Wirral, Formby Nature Reserve (both in Merseyside, NW England) and Brownsea Island (Hampshire, S England) and were compared to samples that had been experimentally cached and uncached samples (see Figure 3.27). Analysis showed no significant differences in work-of-fracture between localities or between wild samples and cached or uncached samples. (Kruskal-Wallis: $H = 8.2$, $p = 0.086$). Norway Spruce cones were not part of the caching experiment, but due to their structural similarity they were compared to Sitka spruce samples, cached and uncached (See Figure 3.28). All Norway spruce samples were collected from Pigeon Wood, Chilterns. No significant difference was detected between spruce samples (Kruskal-Wallis: $H = 0.3$, $p = 0.87$).

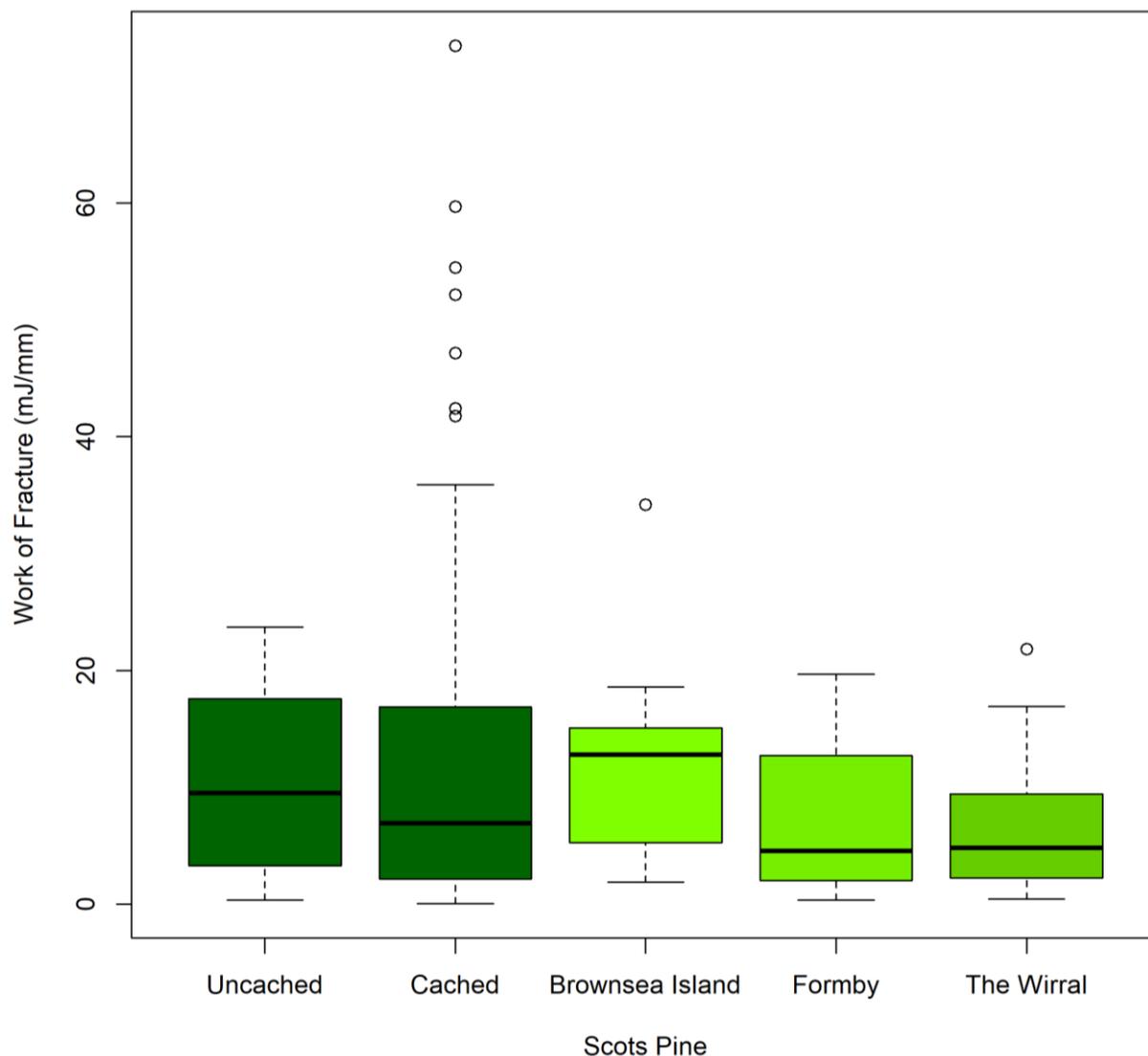


Figure 3.27. Boxplot showing work of fracture/toughness of segments of Scots pine cone scales including those that had not been cached (n = 24), those that had undergone experimental caching (n = 261), and those collected as part of a citizen science project from Brownsea Island (n = 27), Formby Nature Reserve (n = 24) and The Wirral (n = 43). Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.

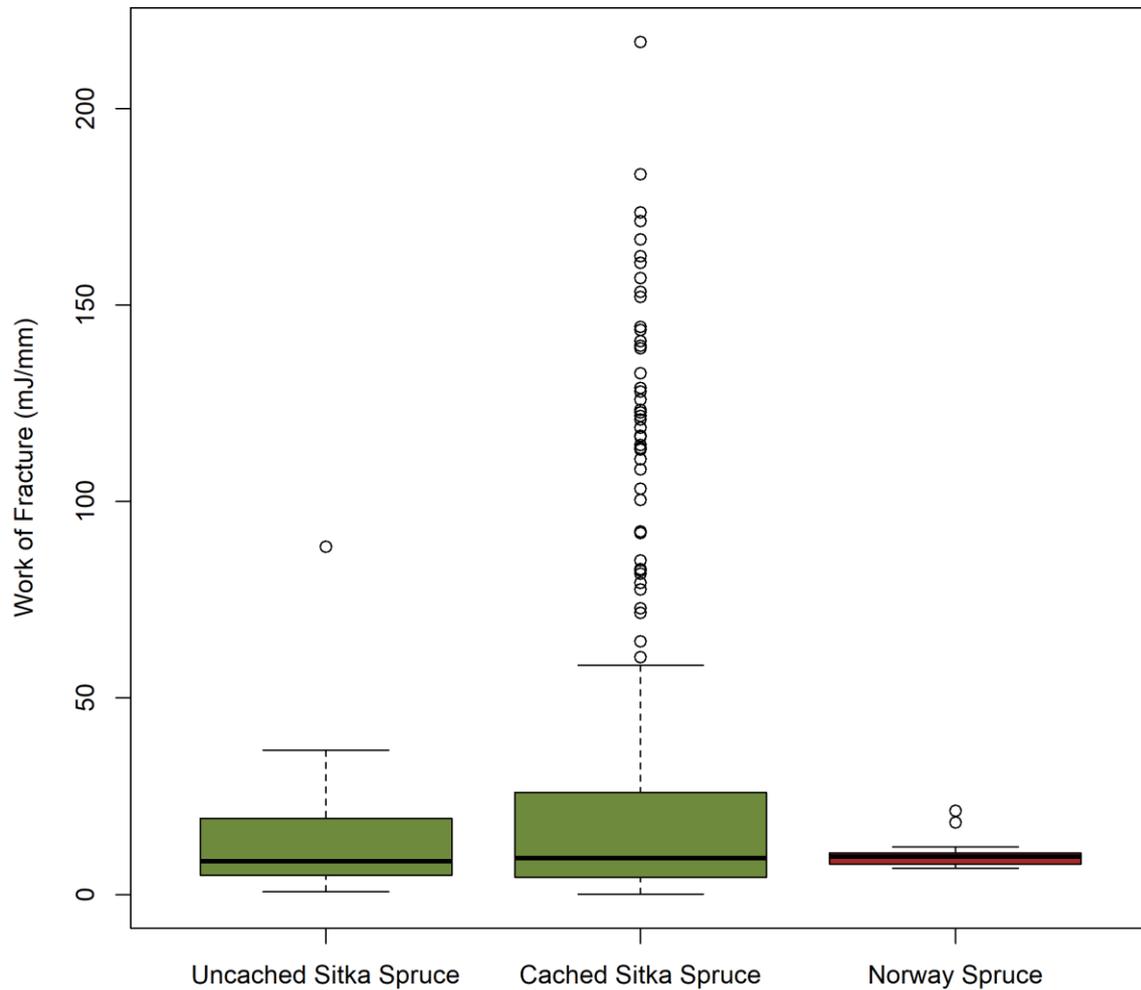


Figure 3.28. Boxplot showing work of fracture/toughness of segments of spruce cone scales including Sitka spruce cones that had not been cached ($n = 30$), those that had undergone experimental caching ($n = 387$), and Norway spruce cones that were collected as part of a citizen science project from Pigeon Wood, Chilterns ($n = 17$). Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.

3.4 Discussion

In this chapter, the aim to create a profile of the dietary mechanical challenges faced by British red squirrel populations has been achieved. Through gathering data on the work-of-fracture (mm/mJ), force to initial fracture (N) and energy to initial fracture (mJ) of eight food items commonly consumed by red squirrels, we can identify which subpopulations are regularly consuming food items that are more mechanically challenging. This allows us to observe the potential link between the food items subpopulations are consuming and the morphology of their masticatory apparatus.

3.4.1. Material properties of food items

The first hypothesis, that the outer casings of supplementary food items consumed by red squirrels (namely peanuts) offer lower work-of-fracture, energy to initial fracture and force to initial fracture than naturally available food items has been supported. Peanut (*Arachis hypogaea*) shells required the lowest work-of-fracture of all species analysed with a mean mJ/mm of 7.61. Although statistical analyses showed this not to be significantly lower than the mean work-of-fracture of hazelnuts, Sitka spruce cone scales or Scots pine cone scales, peanuts also required a relatively low mean energy to initial fracture (135.67 mJ) and mean force to initial fracture (57.75 N). In combination it is therefore likely that peanuts kernels are easiest for squirrels to open, compared to other food items. Beechnuts also required low force and energy to initial fracture, but had relatively high work-of-fracture requirements.

However, it is worth bearing in mind the method in which an animal accesses the kernel. Beechnuts shells have three sides that join at seams, so as to form a structure with a triangular cross section. These seams are points of weakness for the seed, and can quite easily be separated with little force. In practice, squirrels likely avoid cutting through beechnut shells to access the seed, simply accessing the seed by separating the shell surfaces at these points of weakness. Therefore the energy or force to initial fracture is a better representative measure of how difficult the seeds are to access than work-of-fracture, in this case very low. Scots pine and Sitka spruce cones also required relatively low work-of-fracture to cut through the cone scales. Hazelnut shells required surprisingly low work-of-fracture, but the force required to fracture the whole nut was the highest. This demonstrates the importance of looking at all material properties of the food item. Hazelnut shells are relatively hard but brittle, meaning they require a high force input to create the initial fracture. Once the initial fracture is created however, the fracturing is catastrophic. The difficulty for squirrels,

therefore, is creation of that initial fracture. Acorns proved the most difficult to access with high work-of-fracture, energy to initial fracture and force to initial fracture requirements.

3.4.2. Caching

The results of the second hypothesis, that caching reduces the mechanical difficulty of fracture of red squirrel food items, are less conclusive. Caching had a limited but not insignificant effect on work-of-fracture, energy to initial fracture and force to initial fracture among the species analysed. When combining all samples analysed into “cached” or “uncached” samples, beechnuts, sessile oak acorns, hazelnuts and peanuts all showed significant differences in work-of-fracture requirements with caching. For these species, caching actually increased mean work-of-fracture overall except for sessile oak acorn where required work-of-fracture showed an overall decrease after caching. There were no significant differences in energy of initial fracture requirements between uncached and cached samples of the same species, except for beechnuts, where cached samples required less energy to fracture, a pattern which is followed in analysis of force to initial fracture. However, results of caching were largely dependent on the specific variables experienced. For this, it makes more sense to look at each species individually.

Beechnut shells experienced a significant increase in required work-of-fracture when cached. However, no single factor of substrate condition, caching time or temperature appeared to have an effect. Presumably the increase is due to a combination of all three caching conditions. As the ability to access these seeds already requires little force or energy the benefits of caching in terms of material properties is likely negligible. Work-of-fracture required of hazelnut shell showed an overall increase post caching, except in the case of any samples cached in for 3 months which required similar work-of-fracture to uncached samples. For energy to fracture requirements, it was substrate condition that affected overall values, with those samples cached in damp substrate requiring significantly lower energy. Samples cached in no sand or dry sand required similar values to uncached samples. A similar pattern is seen for force to fracture with requirements of samples cached in damp sand being significantly lower than other samples. Generally this would suggest that hazelnut kernels become significantly easier to access when cached in damp conditions for longer periods. English oak acorn shells work-of-fracture requirements did not change significantly with caching, except those cached for 1 week only, which required higher work-of-fracture. Conversely, samples cached for 1 week generally required lower energy and force than

uncached samples. Caching in damp substrate generally increased the requirements but not more than uncached samples, but they were also prone to supporting sprouting of the acorn. This would suggest that English oak acorn kernels are easiest to access when cached for one week in drier conditions. Similarly, work-of-fracture requirements of sessile acorn shells did not change hugely with caching, with results suggesting that samples cached for 3 weeks in damp conditions would require the lowest work-of-fracture values. Energy and force to fracture requirements of whole acorns were more variable with most caching conditions lowering the necessary energy and force to fracture, particularly those cached in damp, warmer conditions. However, samples cached in damp sand, like English oak acorns, were prone to a reduction in edibility with samples in this study largely succumbing to mould. Cached peanuts generally showed an overall increase in work-of-fracture post caching except for those samples cached for 3 months which required similar values. Energy and force of initial fracture of whole peanuts showed that caching, although significant, did not hugely reduce or increase requirements. Work-of-fracture of Sitka spruce cone scales increased overall with caching, with those samples cached for 1 or 3 weeks experiencing a large increase, except those cached in damp sand. Therefore, it is likely easier for squirrels to remove scales from cones either relatively recently removed from the tree, removed from the tree over 3 weeks or kept in damp conditions. Work-of-fracture of Scots pine scales, despite being affected by both time cached and substrate condition, showed limited difference in values between uncached samples and any caching condition.

3.4.2. Mechanical challenges of different diets

The third hypothesis, that squirrels in Formby National Reserve, Merseyside, face lesser mechanical challenges in accessing food items than squirrels in Northern England and Scotland, has also been supported. As discussed in the introduction, British red squirrel populations are highly fragmented, with distinct populations occupying regions with differing habitats. Red squirrels are relatively specific in their diet compared to other squirrel species, such as grey squirrels (*Sciurus vulgaris*) which are generalists. As such, we can narrow down the main food items they are likely to be consuming in specific habitat types. In the Scottish Highlands and South Scotland, where forests are mainly of native Scots pine or introduced timber species such as Norway or Sitka spruce, we can assume that squirrels eat a diet largely of pine seed from these species. This is additionally supported from the literature (Tittensor, 1970; Moller, 1986; Hale & Lurz, 2003). Results from this study therefore indicate the relative difficulty of consuming food items in different populations. In Formby, large

amounts of peanuts have historically been provided to squirrels (Shuttleworth, 1997). Formby is a Scots pine woodland so the other main food source is pine seed (Shuttleworth, 1997). As observed from above experiments, peanuts and pine cones are relatively easily to access, both having comparatively low work-of-fracture values. Additionally, peanuts required low amounts of energy and force to break the outer shell – so whether the squirrel is accessing the kernel through gnawing or cracking it under pure compression, it is relatively easy to consume. Studies have shown in Formby in the 1990s, when peanuts were freely provided throughout the year, peanuts made up to 57% of some squirrels diets (Shuttleworth, 1997). This continuous consumption of one main food source indicates that the diet of the Formby population likely requires the least mechanical requirements. Other populations such as Jersey, where supplementary food such as peanuts are provided to squirrels year round (Magris & Gurnell, 2002), are also likely to require lower bite force. However, in Jersey, acorns are widely available (Magris & Gurnell, 2002), which we know from above experiments are relatively difficult to break open. So the available diet of Jersey is likely overall slightly more difficult to access than Formby.

The Scottish highland populations have access to woodland mostly of native Scots pine seed (Tittensor, 1970; Moller, 1986; Kenward & Holm, 1993) which are shown to be relatively easy to access. Consuming seed from introduced spruce species does not seem to offer significant differences in challenges of accessing food items. So available diet in North Scotland (native woodlands), South Scotland (non-native spruce (Hale & Lurz, 2003)) and Wales (non-native spruce (Shuttleworth, 2015) do not differ much in terms of mechanical challenges. In Northumberland, food items consist of non-native timber plantations of Sitka spruce, Norway spruce and lodgepole pine *Pinus contorta* (Hale & Lurz, 2003). In Brownsea Island, there are both Scots pine and maritime pine *Pinus pinaster* (Thain & Hodder, 2015). As there was limited difference between the conifer species included in this study, we can assume that most conifer species offer similar difficulties in work, energy and force requirements to consume. In Northumberland, beechnuts are also consumed. As discussed above, the work-of-fracture requirements are high for beechnuts, in practice, energy and force to fracture of whole seeds is more important, which was comparatively low. As such we can conclude that the available diets in these regions require middling energy and force inputs to consume, compared to other habitats.

It is squirrels that inhabit deciduous woodlands that likely require the highest work, energy and force to consume their diets. The Isle of Wight is one example, with oak and hazel

woodlands as the main habitat (Kenward & Holm, 1993). However, the density of human habitation on the island, also means there is likely a source of supplementary food available in gardens and parks. In Cumbria, woodlands are mixed, with hazel, oak and yew present but also some non-native spruce (Tonkin, 1983; Hale & Lurz, 2003). Both species of acorn and hazelnuts required relatively high energy and force to crack in pure compression. Acorns also required high work-of-fracture requirements. Hazelnuts too are difficult to access, despite the lower work-of-fracture requirements, the amount force necessary to create an initial fracture is high. Therefore, from analysis, we can say that populations in Cumbria are likely to experience the most energy and force requirements from their diet and the population in Formby is likely to experience the lowest energy and force requirements.

Populations with more mechanically challenging diets are therefore likely to experience selection pressures, with those individuals that can generate the necessary masticatory force and energy to easily access food items, selected for. Populations consistently consuming food items that are less challenging, such as the population in Formby, are unlikely to experience this selection pressure. Results from Chapter 2 demonstrate that the different populations of red squirrels in Britain have variation in the shape, and likely function, of their crania and mandibles. Squirrels in Formby were shown to have mandible and cranial morphology indicative of lesser mechanical abilities, compared to the more robust crania and mandibles of squirrels from other populations. Specimens from Formby collected in the 1990's, when peanuts were provided to squirrels in large quantities, showed the most extreme difference in morphology. We also see a change in morphology between squirrels from the 1990's and the 2010's, pre- and post- removal of peanuts as a supplementary food. Later specimens showed somewhat more robust anatomy, indicative of a higher bite force and greater mechanical abilities. The results of Chapter 3, provides further support that the above variation in morphology is linked to a difference in diet. The reduction in the proportion of peanuts in the diet of Formby squirrels, and a return to consuming more mechanically challenging food items, likely driving a morphological change to more robust masticatory apparatus.

Results of chapter 2 demonstrate that squirrels in Northern England possess the most robust masticatory apparatus, and this coincides with a diet of the most difficult food items to access. The mixed diet of Cumbria and Northumberland squirrel populations, is more mechanically challenging and therefore squirrels in these areas will require higher bite forces and greater mechanical abilities. While not as robust as Northern English squirrels, squirrels in North Scotland were also shown to have robust anatomy suggesting an ability to generate

higher bite forces than squirrels from Formby. While the diet of squirrels in these populations is largely Scots pine and Sitka spruce seeds, which ranked quite low in toughness and therefore should be easily accessible, we should consider the overall differences in consuming a diet of cones compared to a mixed diet. Pine seeds are much smaller than hazelnuts or acorns. As such, squirrels must consume a higher quantity of pine seeds to gain the same nutritional value of a single hazelnut. Each pine seed is protected by a single scale. To remove all seeds from a single cone would require repeated action of removal of cone scales. This is time consuming and overall requires a higher energy cost than a single larger seed such as a hazelnut. Squirrels in Jersey had mandibular morphology most similar to Formby specimens, suggesting an overall lower bite force, which may correlate to the high proportion of supplementary food received by squirrels on the island (Magris & Gurnell, 2002).

3.4.4. Further Work

In selecting the three different parameters in this study, work-of-fracture, force to initial fracture and energy to initial fracture, it was intended to capture a range of material properties of the different food items for comparison. Results of energy and force to initial fracture demonstrate the difference in material properties between whole nuts placed under pure compression. It is unlikely that an animal like a squirrel would ever be placing food items of this size under pure compression. Therefore, work-of-fracture was also measured to more accurately reflect what squirrels may actually experience (and also allowing cones to be included in the analysis). Work-of-fracture or 'toughness' places a quantitative measure on the difficulty of propagating a crack in the shell of nuts. Creating and widening a crack in the shell is the method necessary for a small mammal such as a squirrel to succeed in opening the shell to retrieve the edible kernel inside. This is likely the more important parameter that a squirrel would need to overcome. But there are other considerations to how the food items are consumed. Rodents all 'gnaw' their food which is a repetitive action. This likely avoids the need to apply high amounts of force in a single bite, instead wearing away at the seed shell, creating a point of weakness where it will be easier to create to fracture. In cones, the scales must be cut through to remove them from the cone and then pulled away. With so many scales to remove from the cone, the repeated action of scale breakage and removal is likely energy consuming, and the muscles involved in this action are probably different or used in different ways than the actions of gnawing or biting. Squirrels also use their forepaws to

manipulate the seeds to get to the best areas of weakness. Therefore, more research is needed to investigate how squirrels use their anatomy to access food items.

3.4.4. Limitations

The caching experiments performed in this study were a necessary oversimplification of real world conditions. Often seeds are cached by squirrels in a number of places, unearthed and recached. It is unlikely that they will be stored in conditions with consistent temperatures like this study. Buried food items will be subject to the work of other organisms such as plants, fungi and invertebrates which likely further damage the food items. Soils differ majorly between localities with pH and moisture retention among just some of the factors that may affect the mechanical properties of cached food items. However, the number of factors that could be considered are too vast and varied to be within the remit of this study. Repeats of this experiment that are more accurate could be performed where different soils samples are used from different localities and a wider range of time scales are used. Regardless, the above results can be considered a pilot study in terms of the effects of caching on the material properties, and edibility, of seeds.

Additionally, while this study aimed to be comprehensive of the food items consumed by red squirrels in Britain some food items were not included. Tree species generally differ across the country and some species were not considered common enough to be included in this analysis. Sweet chestnuts is one example of this. Squirrels are generally opportunistic and will consume other food items when provided those. There are many 'secondary foods' that squirrels regularly consume to supplement their main diet of nuts and seeds (Shorten, 1954; Moller, 1983). Further research may wish to include other food items within the analysis. However, for the specifics of this chapter those included represented the general diets overall. It is also worth noting that, while we can assume a general diet of a population by observing what food items are locally available, we cannot definitely state what food items a specific squirrel consumes. However, isotopic analysis has proved useful in allowing researchers to see general trends in food items consumed. This has been employed in the publication of results of Chapter 2 (Chandler et al. In Review), where $\delta^{15}\text{N}$ Nitrogen ratios of bone collagen revealed that the Formby 1990s population had a significantly different diet than other populations in the study.

3.4.5. Conclusions

The results of this study demonstrate that food items across a populations range can offer varying mechanical challenges to different subpopulations of the same species. This research provides further support to the hypothesis that material properties of food items is driving morphological change in the masticatory apparatus of British red squirrel populations. However, this study creates questions as to how the morphological changes affect the function of the masticatory apparatus. We know that there is a difference in jaw shape across British populations and this correlates with a difference in mechanical properties of food items, however how exactly (and whether) these changes in shape correspond to a change in function, adapting to better able to deal with differing mechanical challenges, remains to be seen.

4. Investigation into Biomechanical Function of the Red Squirrel Mandible using Finite Element Analysis

4.1 Introduction

The concept of bone functional adaptation is a principle, based on Wolff's law, whereby organisms are capable of adapting their physical structure to new living conditions, and that bone cells can respond to locally applied mechanical strain (Frost, 1994). A simple feedback loop explaining this is set out in Lanyon & Skerry (2001), where bone deposition by osteoblasts occurs under increased strain and bone resorption by osteoclasts occurs under decreased strain. This allows overall maintenance of an optimum strain level in the structure. As bones serve as the structures upon which muscles attach, the morphology of bone is significantly impacted by the use of muscles (Frost, 1994). Muscles that are used frequently will likely increase the amount of strain experienced by the attached bony structure, while under-used muscles are likely to impart lower strain (Frost, 1994). The increased bone deposition resulting from elevated strain levels increases bone volume, thus reducing overall strain. This functional adaptation means the morphology of an animal can change significantly within its lifetime (Frost, 1994). Analysing the differences between the morphology of key bony structures of individual animals within a species can reveal different behaviours, lifestyles or diets between populations (e.g in Cox et al., 2020; Doudna & Danielson, 2015; Franssen, 2011; Yazdi, 2011) Furthermore, analysing the relative strains and stresses experienced by bony structures of an organism can indicate how the individuals are using parts of their anatomy, and how the functional abilities of a structure can differ across a population or species.

The mandible is an ideal skeletal structure to study the link between morphology and function, as in most animals there is only one function of the mandible, mastication. This removes the need to consider other, potentially conflicting, functions that may be environmentally selected for, when inferring the functional abilities from the morphology of the mandible. In Chapter 2, the biggest differences in morphology were detected within the mandible. Many studies have indicated that rodent mandible shape is sensitive to changes of the relative hardness of food items (Anderson et al., 2014; Renaud et al., 2015; Renaud & Auffray, 2010). From our understanding of bone functional adaptation, a reduction in the amount, or strength of, muscle contraction needed in the mastication of a soft diet, imparts a reduction in the amount of bone deposition occurring. The opposite is true of mastication of

hard diets, which require higher muscle forces, increasing the load on the mandible and increasing rates of bone deposition. Within lifetime morphological change of the masticatory apparatus has been observed in experimental studies on rats (Mitchell et al., 2021; Ödman et al., 2008; Tanaka et al., 2007), mice (Enomoto et al., 2010), rabbits (Menegaz et al., 2010), ferrets (He & Kiliaridis, 2003) and primates (Corruccini & Beecher, 1982), with some studies linking morphological differences with functional variation (Mitchell et al., 2021).

Experimental studies have shown that mice or rats fed soft food diets had lower mechanical advantage (Anderson et al., 2014), reduced bone mineral density (Mavropoulos et al., 2005) or reduced bone volume (Enomoto et al., 2010) of the mandible. Similar forms of adaptive plasticity of the rodent mandible have also been seen in wild populations with access to different diets (Casanovas-Vilar & van Dam, 2013; Doudna & Danielson, 2015; West & King, 2018).

Rodents have distinct dentition of enlarged incisors in both upper and lower jaws. The rodent mandible and cranium are of different lengths, prohibiting the simultaneous occlusion of both the incisors and the molars. Rodents therefore have two feeding modes that are mutually exclusive, incisor gnawing and molar grinding. They switch between modes through anterior or posterior movement of the mandible, termed propaliny (Becht, 1953; K. M. Hiiemae & Ardran, 1968). This unusual dentition and the need for propalinal sliding requires highly specialised musculature. In rodents, the main muscles of mastication are the temporalis muscle and masseter muscles. The masseter is the dominant muscle of mastication, divided into three layers: the superficial masseter, deep masseter and the zygomaticomandibularis (Cox & Jeffery, 2015). Almost all extant rodents have one of three distinct morphologies termed hystricomorphy, myomorphy or sciuromorphy (Wood, 1965; Cox et al., 2012). Squirrels are sciuromorphs, and therefore have a deep masseter that extends forwards onto the rostrum, taking its origin underneath the widened anterior root of the zygomatic arch (Cox et al., 2012). The use of the muscles of mastication are different for the two modes of mastication, with analysis of the rodent cranium suggesting different patterns of stress and strain across the cranium during each mode (Cox et al., 2012). Sciuromorphy is particularly well suited for incisor gnawing, being more efficient at translating muscle force into bite force at the incisors than hystricomorphy or myomorphy (Cox et al., 2012; Druzinsky, 2010b).

The muscles of mastication are dependent on the support of the bone regions to which they attach (Baverstock et al., 2013). In the rodent cranium, both the deep masseter and the

zygomaticomandibularis are attached to the zygomatic arch, therefore this region experiences the largest stresses during biting (Cox et al., 2012). In the mandible, the angular process is the attachment site for the masseter muscles so it is here we would expect the highest levels of stress. The condyle enables interaction between the cranium and the mandible and the coronoid process is the attachment site for the temporalis (West & King, 2018).

In Chapter 2, we see that the morphology of Formby red squirrels from the 1990s is distinctly different from other British red squirrel populations. Comparatively, specimens from Formby 1990s have longer, narrower skulls with a flatter cranial vault and a ventrally positioned temporalis scar. The mandible is also narrower with a curved coronoid process and shallow inflection between the condylar and angular processes. The flatter cranial vault coupled with a more ventrally positioned temporalis scar suggests an overall smaller temporalis muscle. The more acute angle between the coronoid process and the condyle potentially results in shorter ventral temporalis muscles fibres. From this we can deduce that the functional ability of certain muscles in squirrels of this population will be significantly affected. Previous studies suggest that a shorter, broader mandible, such as that in the Borders populations of red squirrels, can apply greater mechanical force, as the attachment site for the masseter muscle is increased (Michaux et al., 2007; Satoh, 1997). To determine whether the morphological differences between populations of red squirrels are indeed affecting the functional abilities of their masticatory apparatus, further biomechanical analysis needs to be explored.

Analysing biomechanical performance can be done using Finite Element analysis (FEA), a computational engineering technique where behaviours or actions are simulated on digital renders of scanned specimens. (Bright, 2014; Panagiotopoulou, 2009; Rayfield, 2007; Richmond et al., 2005). Investigations of cranial mechanics are often explored using FEA, with some of the first studies using this technique looking at feeding (Rayfield, 2004). Muscles can be digitally mapped on the models, along with the amount of muscle force generated and the direction of contraction.

Performing these simulations allows us to then extract values for stress and strain. Principal strain 1 and principal strain 3 approximate the tensile strain and the compressive strain experienced across a structure respectively. These strains can also be referred to as 'maximum principal strain' and 'minimum principal strain' respectively. As the extreme ends of the range of strain values is where bone remodelling will take place, these strains are most

important to analyse. Principal strain 2 is shear strain, which is less informative for this study. von Mises stresses indicate how and where the structure is close to its yield point. Using extracted values we can also calculate the mechanical advantage. It is assumed that there is a selective advantage for morphology that lowers the overall von Mises stress across the mandible, distributing the stress so no points across the mandible are likely to experience fracture when loaded. We can then predict which model mandibles ‘perform better’ under load, with the assumption that mandibles experiencing the lowest overall stress have the highest protections against structural failure. Mechanical advantage is a measure of mechanical efficiency of the mandible calculated as the ratio of output bite force to input muscle force. It represents the proportion of muscle force translated into bite force and not lost in deformation of the mandible or generation of joint reaction forces. (Dumont et al., 2011). Mechanical advantage is also a convenient measure for comparison across models, to compare biting efficiency. Another advantage of FEA is that parts of the anatomy can be removed or manipulated at will, avoiding the practical or ethical concerns that would be of consequence in *in vivo* work. This method, which I will refer to as virtual ablation (as in Cox et al., 2013; Cox & Jeffery, 2015; Cox, 2017), can be useful in determining the relative stress or strain imparted onto structures by specific muscle groups. While the function of the masticatory apparatus of many animal species has been analysed through FEA (Cox et al., 2012; Cox et al., 2011), limited studies have been performed on the mandible of multiple individuals of the same species.

4.1.1 Aims

This chapter aims to establish, with the use of FEA, the biomechanical performance of the red squirrel mandible across four populations. The goal of this is to investigate whether the shape variation of British red squirrel populations, detailed in Chapter 2, is evidence of different selective pressures for functional abilities, posed by hard or soft food diets (as detailed in Chapter 3). Additionally, the effect of morphological change in the mandible on the soft tissues of mastication, namely the temporalis, superficial and deep masseter muscles, is investigated. Other factors may impact feeding biomechanics of red squirrels (such as muscle mass, fibre length, pennation angle of fibres, tooth sharpness and gape angle), however these are outside of the scope of this study which will focus on mandible shape only.

4.1.2 Hypotheses

1) *When loaded to simulate incisor biting, the strain (principal strain 1 & 3) and stress (von Mises) experienced by the mandible model of the 1990s Formby specimen will be higher than that of mandibles models from the 2010s Formby, Northern England and Northern Scottish populations. This is dependent on the assumption that the softer food diet of the 1990s Formby specimens has removed the selective pressure for a mandible capable of performing well under high loads, therefore leading to bone resorption, owing to the lower strain environment.*

2) *When loaded to simulate molar biting, the relative strain and stress experienced by the mandible will show differences between populations. As the main difference between the hardness of food items is the shell in which they are stored, for which incisor biting/gnawing is employed to fracture, the resistance to stress and strain may not be as actively selected for as incisor biting. However, the difference in morphology of the mandible between populations, driven by different rates of bone deposition and resorption, and therefore the different models, will have some overall effect on how well the mandible performs under molar loading.*

3) *Mechanical advantage (calculated as the total bite force divided by the total muscle force) of 1990's Formby specimens will also be lower than that of other specimens. As mechanical advantage is a measure of the proportion of the muscle force translated into bite force, we would expect higher mechanical advantage in those populations with a selection pressure for high bite forces. As individuals from Formby 1990s population consume a less mechanically challenging diet, the selection pressure is removed and we would expect the mechanical advantage to be comparatively lower.*

4) *Virtual ablation of the masseter and temporalis muscles will remove most differences in stress and strain between specimens, as these are the muscles primarily affected by shape changes between specimens.*

4.2 Materials and Methods

4.2.1 Samples and Model Reconstruction

The mean hemi-mandibular shape of a dataset of four populations of British red squirrels (North Scotland, Borders, Formby 1990s and Formby 2010s) was calculated using geometric morphometrics (see Chapter 3). One specimen closest to the mean for each population was selected for microCT scanning (see specimen list below). Specimens were loaned from the National Museum of Scotland and scanned at the XTM Facility, Palaeobiology Research Group, University of Bristol with a Nikon XT H 225 ST CT scanner. Voxels were isometric, dimensions per specimen listed in Table 4.1 below.

Table 4.1. List of specimens, calculated as closest to the mean shape of that specific population, selected for microCT scanning.

Specimen No.	Population	Collection Date	Sex	Hemimandible Length**	Original Voxels	Resampled Voxels
NMS 2000.194.045	Formby	1995	Male	3.21 cm	21 μm	84 μm
NMS SR106.20*	Formby	2010 - 2020	Unknown	3.28 cm	28 μm	58 μm
NMS 2000.195.69	North Scotland	1988	Male	3.10 cm	28 μm	59 μm
NMS 2000.194.031	Borders	1995	Female	3.14 cm	29 μm	59 μm

*Temporary museum number. **Where length is measured as the shortest distance between the tip of the condylar process and where the incisor tooth erupts from the mandible.

Three dimensional reconstructions of the hemi-mandibles were created in Avizo Lite v9.2 (Thermo Fisher Scientific, Waltham, MA, USA) through automated and manual thresholding. In order to reduce processing time, the models were down-sampled to voxel sizes detailed in Table 4.1 above. Bone, incisor teeth and molar teeth were segmented as separate volumes to allow for assignment of differing elastic properties. Previously published sensitivity analyses show limited difference in strain patterns between models that distinguish trabecular and cortical bone and those that model all bone as cortical (Fitton et al., 2015; Toro-Ibacache et al., 2016). Hence, cancellous bone of the hemi-mandibles had all holes and gaps filled in

digitally and the structure treated as if it were solid cortical bone. Additionally, pulp cavities of incisors and molars were filled and assigned the same material properties of the associated teeth to create solid structures. Gaps between bone and teeth, in life occupied by tissue (e.g. periodontal ligaments), were also filled and assigned the same material properties as bone. Removal of the periodontal ligament is unlikely to affect overall strain patterns, with previous work demonstrating that inclusion only shows localised differences in strain patterns around the tooth alveoli (Wood et al., 2011). All models were mirrored, reflected and merged into a single mandible. To ensure correct positioning, the mandibular condyles were aligned with the glenoid fossae of the crania of that specimen, 3D models of which were collected as 3D surface scan data (see Chapter 2).

4.2.2 Conversion to FE Model

Reconstructions were converted to an 8-noded cubic mesh from voxels using VOX-FE (J. Liu et al., 2012). Material properties assigned to cortical bone, incisor teeth and molar teeth were based on previously published data for rodent teeth and bone (Williams & Edmundson, 1984; Cox et al., 2011), see Table 4.2, below. Separate values of Young’s Modulus and Poisson’s ratio were assigned for incisors and molars, due to different proportions of enamel and dentine. Values for incisors were calculated as an average of the material property values for rodent enamel and dentine (Cox et al., 2011; Morris et al., 2022). All models were assigned the same material properties.

Table 4.2. Material properties assigned to FE models.

	Young’s Modulus	Poisson’s Ratio
Cortical Bone	17 GPa	0.3
Incisor Teeth	55 GPa	0.32
Molars Teeth	30 GPa	0.3

Mandibles were constrained to prevent translation or rotation in space. Left and right condyles were constrained in the x, y and z axis to simulate the temporo-mandibular joint. Additionally, the tooth of interest (or ‘biting’ tooth) was constrained perpendicular to the occlusal plane. The number of nodes constrained at each location ranged between 157 - 282 nodes for condylar constraints, 48 -101 for incisor constraints and 104 – 107 for molar

constraints. Seven muscles of mastication were modelled on each side of the mandible based on anatomical descriptions and diagrams from Ball & Roth, 1995; Thorington & Darrow, 1996 and Druzinsky, 2010.

A commonly used method to calculate muscles force values for application to the models, is the physiological cross-sectional area multiplied by a muscle stress value of 0.3 Nmm⁻² (e.g. (Cox & Watson, 2023). As the physiological cross-sectional area of the relevant muscles is usually obtained from dissections, and this was not available for the specimens used in this study, estimates must be made. Cox and Watson 2023 dissected a red squirrel from Formby post-2010 (NMS GH.209.22). The physiological cross-sectional area of the muscles of this specimen were used to calculate the muscle forces for the Formby post-2010 mandible in this study (Specimen NMS SR106.20), as they are likely to be most morphologically similar to each other. For the other specimens in the study, the muscle forces were scaled to the surface area of the other three specimens, in order to remove any potential differences in size between muscles of the specimen in Cox and Watson 2023 and those in this study. Scaling was performed using the following equation, where A is one of the other models (NMS 2000.194.045, NMS 2000.195.069, NMS 2000.194.031), B is the Formby post-2010 model (NMS SR106.20), SA is surface area and F is muscle force. Details of muscle forces applied are listed in Table 4.3 below.

Table 4.3. Muscle forces (N) per single muscle applied to models after scaling to same force per area as Formby 2010s model.

Muscle	Original Force	Formby 1990s	Borders	North Scotland
Ant Deep Mass	7.1	7.580	6.741	7.140
Pos Deep Mass	6.8	7.260	6.456	6.839
Superficial Mass	6.7	7.153	6.361	6.738
ZMM	5.5	5.872	5.222	5.531
Temporalis	5.7	6.086	5.412	5.732
Med Pterygoid	5	5.338	4.747	5.028
Lateral Pterygoid	3.7	3.950	3.513	3.721
Hemi-mandible Total	40.5	43.240	38.451	40.729

3D models of the cranium associated with each specimen were available for muscle orientations, which were determined by creating a vector between the muscle origin on the mandible and the insertion site on the cranium. Landmarks were placed at the centroid of each muscle attachment site on the crania in Avizo and then uploaded into VOX-FE to provide end points for vectors. Muscle orientations were adjusted depending on which tooth was under load, to account for the propalinal movement of the mandible that occurs between molar and incisor feeding modes of rodents. To subject models to loading scenarios simulating both incisor biting and molar biting, constraints were placed on the tip of both incisors, or the centre point of the M1 respectively (see Figure 4.1).

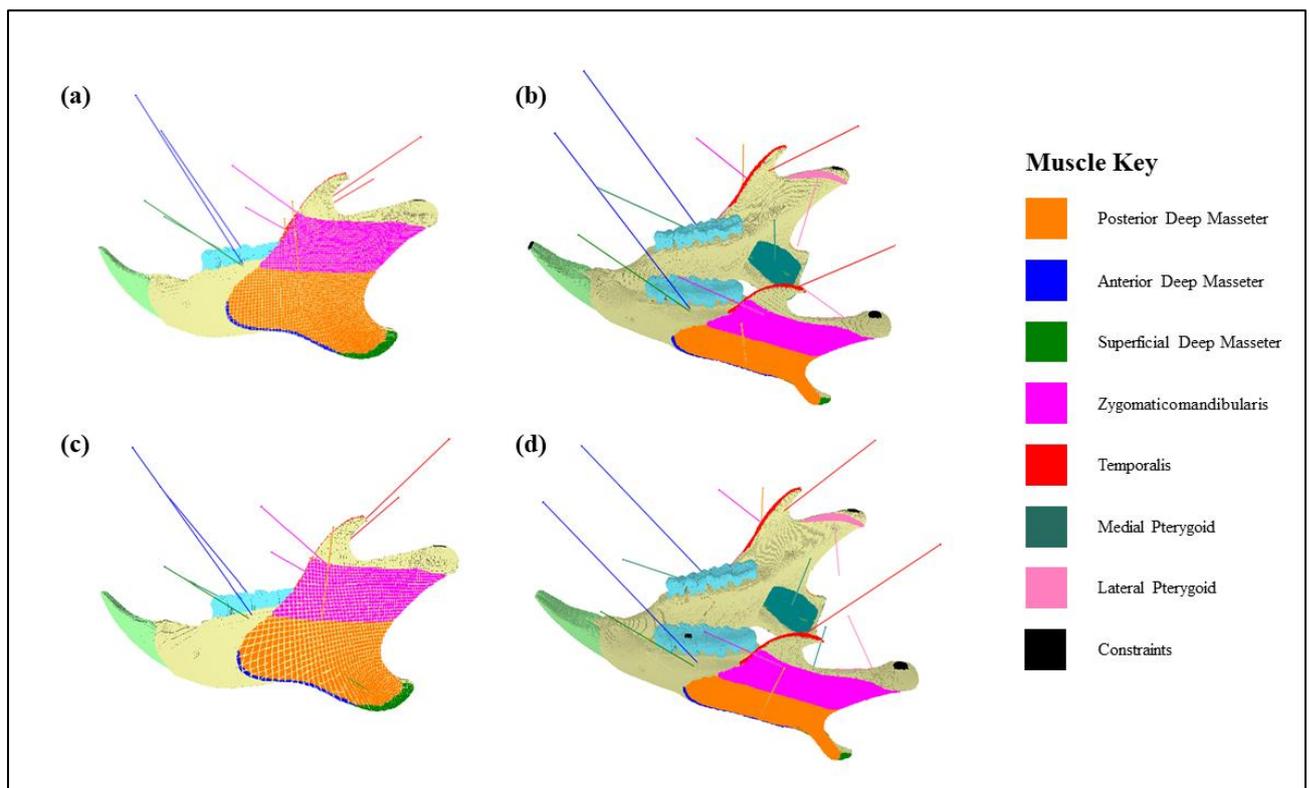


Figure 4.1. Muscles and muscle orientations modelled on specimen NMS 2000.194.031, based on anatomical descriptions and diagrams from Ball & Roth, 1995; Thorington & Darrow, 1996 and Druzinsky, 2010. Image (a) and (b) demonstrate vectors between the muscle origin on the mandible and the insertion site on the cranium during incisor biting, while (c) and (d) demonstrate vectors between the muscle origin on the mandible and the insertion site on the cranium during molar chewing.

4.2.3 Virtual Ablation

The temporalis, deep and superficial masseter muscles were identified as muscles most likely to be affected by morphological changes of the mandible bone, identified in Chapter 2. In order to establish whether these muscles play a significant role in the patterns of stress and strain experienced by the models, and therefore the biomechanical performance of the mandibles, each mandible model was re-analysed with specific muscles ‘switched-off’. To maintain the overall input muscle load, the force of the removed muscles were redistributed proportionally across the other muscles in the model. This resulted in four sets of results for each mandible, one with all muscles ‘switched-on’, one without a temporalis muscle, one without a deep masseter muscle and one without the superficial masseter. Muscle forces applied to the models in absence of specific muscles are given in Table 4.4 below.

Table 4.4. Muscle forces (N) per single muscle applied to models with virtual ablation of specific muscles. Total Muscle force totals muscles applied to both hemi-mandibles.

Muscle Virtually Ablated	None	Temporalis	Deep Masseter	Superficial Masseter
Anterior Deep Masseter	7.1 N	8.3 N	-	8.5 N
Posterior Deep Masseter	6.8 N	7.9 N	-	8.1 N
Superficial Masseter	6.7 N	7.8 N	10.2 N	-
Zygomatocmandibularis	5.5 N	6.4 N	8.3 N	6.6 N
Temporalis	5.7 N	-	8.6 N	6.8 N
Medial Pterygoid	5 N	5.8 N	7.6 N	5.9 N
Lateral Pterygoid	3.7 N	4.3 N	5.6 N	4.4 N
Total Muscle Force	81 N	81 N	81 N	81 N

4.2.4 Model solution and analysis

Values of von Mises stress, tensile strain (principal strain 1), compressive strain (principal strain 3) were output from FE analysis. Maximum bite force generated by each model could be calculated from the force output file of the model solution, calculated as the sum of force experienced at the constraint on the incisor/molar tooth. This could then be used to calculate the mechanical advantage by dividing the bite force by the total input muscle force for each model.

To accurately compare experienced stress and strain between models, output values for median stress and strain were scaled to represent what each model would be experiencing while generating the same bite force. The calculated maximum bite forces from FE analysis of each model were roughly around 20 N for incisor biting and 40 N for molar biting, therefore values of median stress and strain were scaled to produce a incisor bite force of 20 N and a molar bite force of 40 N (see Table 4.4 for results of bite force before scaling). To calculate the scaled results, the original maximum bite force was divided by the new bite force to create a scaling factor for each model. The relevant scaling factor was then multiplied by the median values of stress and strain for each model. This follows methodology used in Fitton et al. 2012, 2015. Median values were chosen as this most accurately represents the experience of most finite elements in the structure. Choosing mean values allow for heavy influence of extreme outliers which are sometimes the case due to artefacts in the model.

Patterns of stress and strain across the mandible are visualised in colour maps. Scaling was also used to ensure the colour maps were also showing stress and strain at the same bite forces across the models. To show a colour map of max display range 400 μ strain, the max display range is set at 400 divided by the relevant scaling factor for that loading scenario.

4.2.5 Geometric Morphometrics

Deformation patterns across the mandible models under both incisor and molar loading were analysed using geometric morphometrics. Loaded and unloaded models were landmarked following the three-dimensional landmark configuration in Figure 4.2 below, and values of deformation were extracted at each landmark. Landmarks were copied from the geometric morphometrics study in Chapter 2 (see Section 2.2.1 *Landmarking*) as these landmarks accurately captured previous shape variation in the mandible. The landmarks were subjected to generalized Procrustes analysis and scaled to centroid size. The residual difference between the loaded and corresponding unloaded models were then added to the landmark configuration of the unloaded models. A second generalized Procrustes analysis was then performed on this new set of landmarks, without scaling. As size change is an important component of the deformation that we want to measure, we do not want to remove size differences between models. This was then followed by principal component analysis (PCA) on the Procrustes coordinates, summarising mandibular deformation from the mean shape

along the main axes of the morphospace. The Procrustes distances between models and the mean model was calculated.

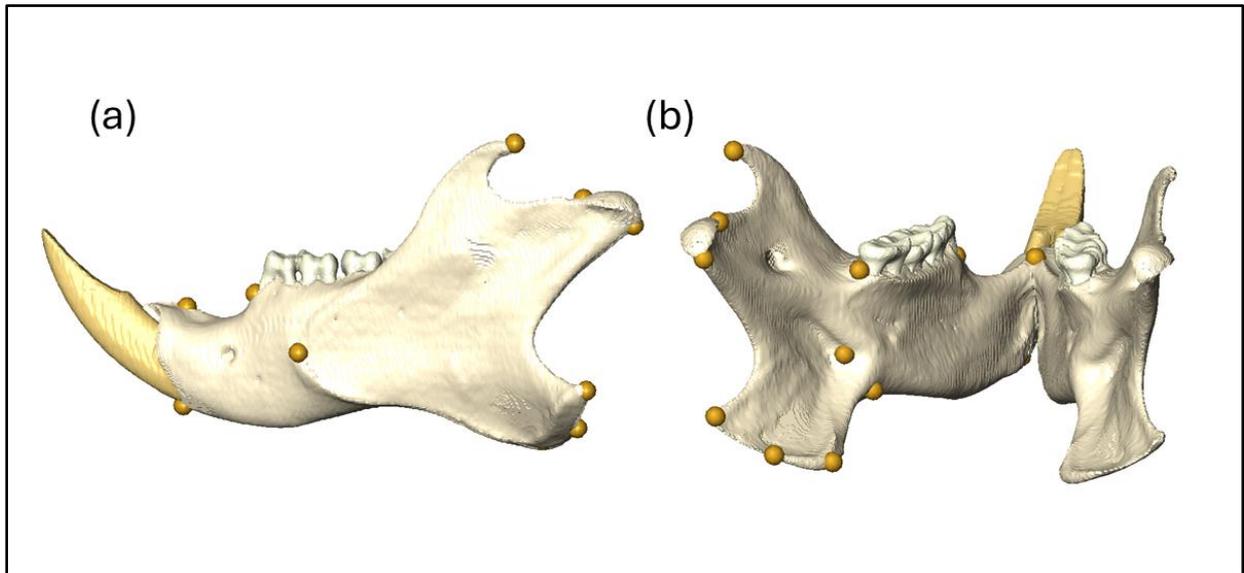


Figure 4.2. Landmark configuration represented on specimen NMS 2000.194.045 (a) left lateral and (b) anterior view.

4.3 Results

4.3.1 Strain and Stress under incisor loading

Similar patterns of stress and strain are seen across all mandibles placed under incisor loading, with some differences (see Figure 4.3). High values of principal strain 1, which approximates tension, are concentrated around the condylar, angular and coronoid regions as well as the diastema. The Formby 1990s specimen appears to have slightly lower values in the region between the condyle and the angular process compared to other specimens. Median values of principal strain (see Table 4.5) show that overall the Formby 1990s specimen experienced the lowest overall tensile strain while North Scotland experienced the highest. Across all models, high values of compressive strain, approximated by principal strain 3, are concentrated around the condyle and the middle of the ramus. A zone of high strain connects these two areas in all models except for the Formby 1990s specimen. Overall median values of principal strain 3 are lowest for the Formby specimen and highest for the Formby 2010s specimen. Patterns of von Mises stress reveal that the models are close to their yield point around the posterior portion of the mandible, particularly around the condyle but also in the diastema. The Formby 1990s population appears to experience lower von Mises stress, than other populations, supported by the median values.

Table 4.5. Median Values for Principal Strain 1, Principal Strain 3 and Von Mises Stress experienced under incisor biting loading, for four models of red squirrel mandibles.

	Principal Strain 1 (μstrain)	Principal Strain 3 (μstrain)	Von Mises Stress (MPa)
Formby 1990s	141.2	-130.1	6.2
Formby 2010s	155.3	-144.1	6.5
Borders	159.3	-150.3	7.0
North Scotland	171.5	-160.4	7.1

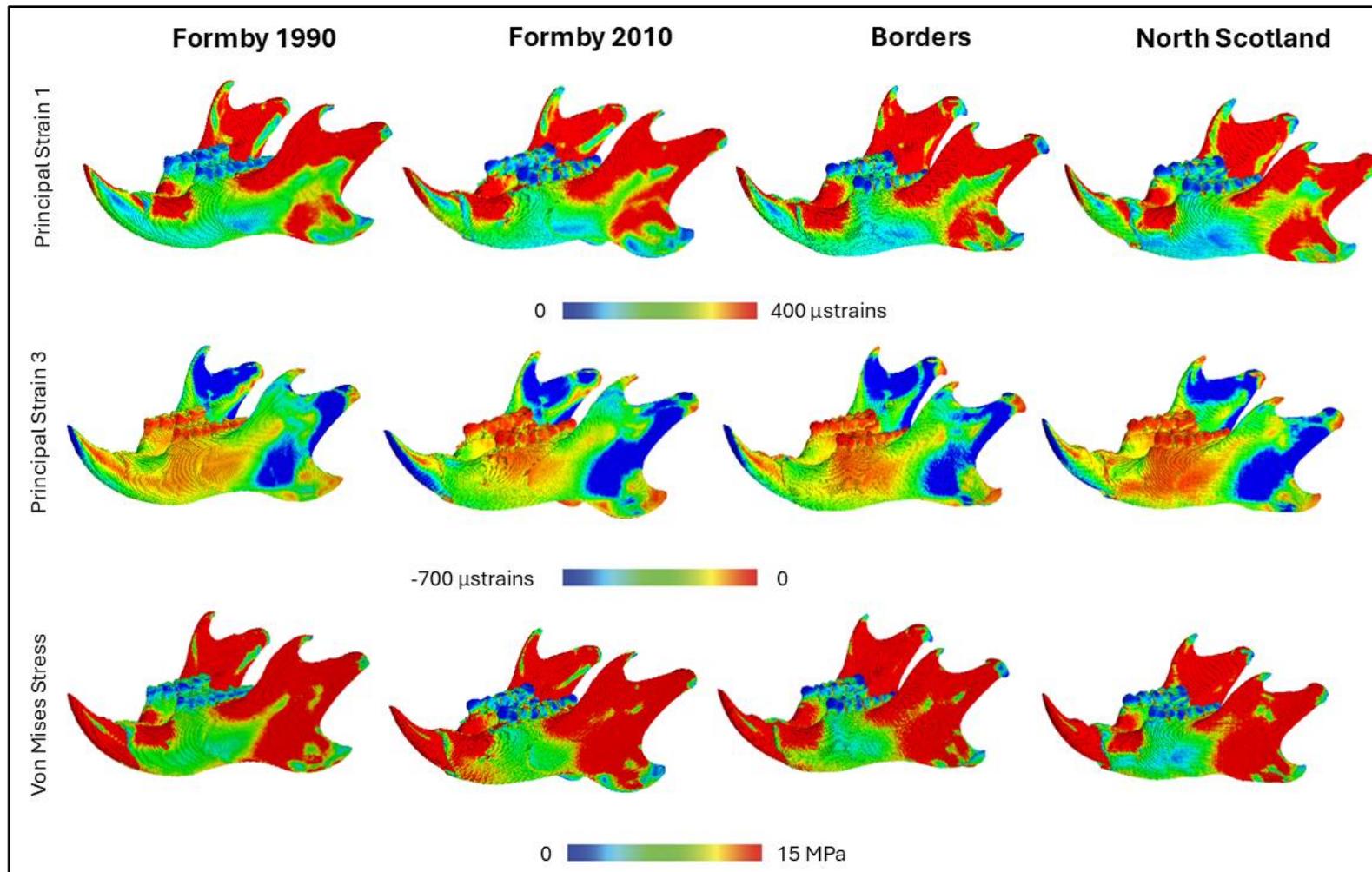


Figure 4.3. Colour maps of Principal Strain 1, Principal Strain 3 and von Mises stress across models of red squirrel mandibles under incisor biting scenarios. Principal Strain 1 (tensile strain), Principal strain 3 (compressive strain) and Von Mises stress show highest strain in warm colours and lowest strain in cool colours. All colour maps were scaled to represent a maximum bite force of 20 N.

4.3.2 Strain and stress under molar loading

Although similar overall patterns for stress and strain are seen across all mandibles for molar loading, the differences are more pronounced than the differences between models under incisor loading (see Figure 4.4). High values of tensile strain (principal strain 1) are seen mainly around the coronoid process and the masseteric ridge. In the Formby 2010s specimen, this highly strained region extends between these two areas. This is supported by median values for principal strain 1 which show overall tensile strain to be highest in the Formby 2010s model and the lowest in the Formby 1990 model (see Table 4.6). There were few areas of high compressive strain in any of the models, with most compressive strain experienced in the medial upper ramus, between the coronoid and the condyle. Patterns of von Mises stress of mandibles under molar loading are similar to patterns of principal strain, with areas of high stress concentrated in the coronoid, border of the mandible body and also extending onto the diastema. The Formby 2010 specimen experienced higher stress with areas of high stress extending down the ramus onto the mandible body. The Formby 1990s specimen experienced the lowest overall von Mises stress with areas of high stress concentrated on the coronoid, posterior condyle and the diastema only.

Table 4.6. Median Values for Principal Strain 1, Principal Strain 3 and Von Mises Stress experienced under molar biting loading, along with calculated mechanical advantage, for four models of red squirrel mandibles.

	Principal Strain 1 (μstrain)	Principal Strain 3 (μstrain)	Von Mises stress (MPa)
Formby 1990s	113.9	108.7	4.9
Formby 2010s	130.3	129.2	5.8
Borders	134.4	123.8	5.7
North Scotland	121.6	122.4	5.6

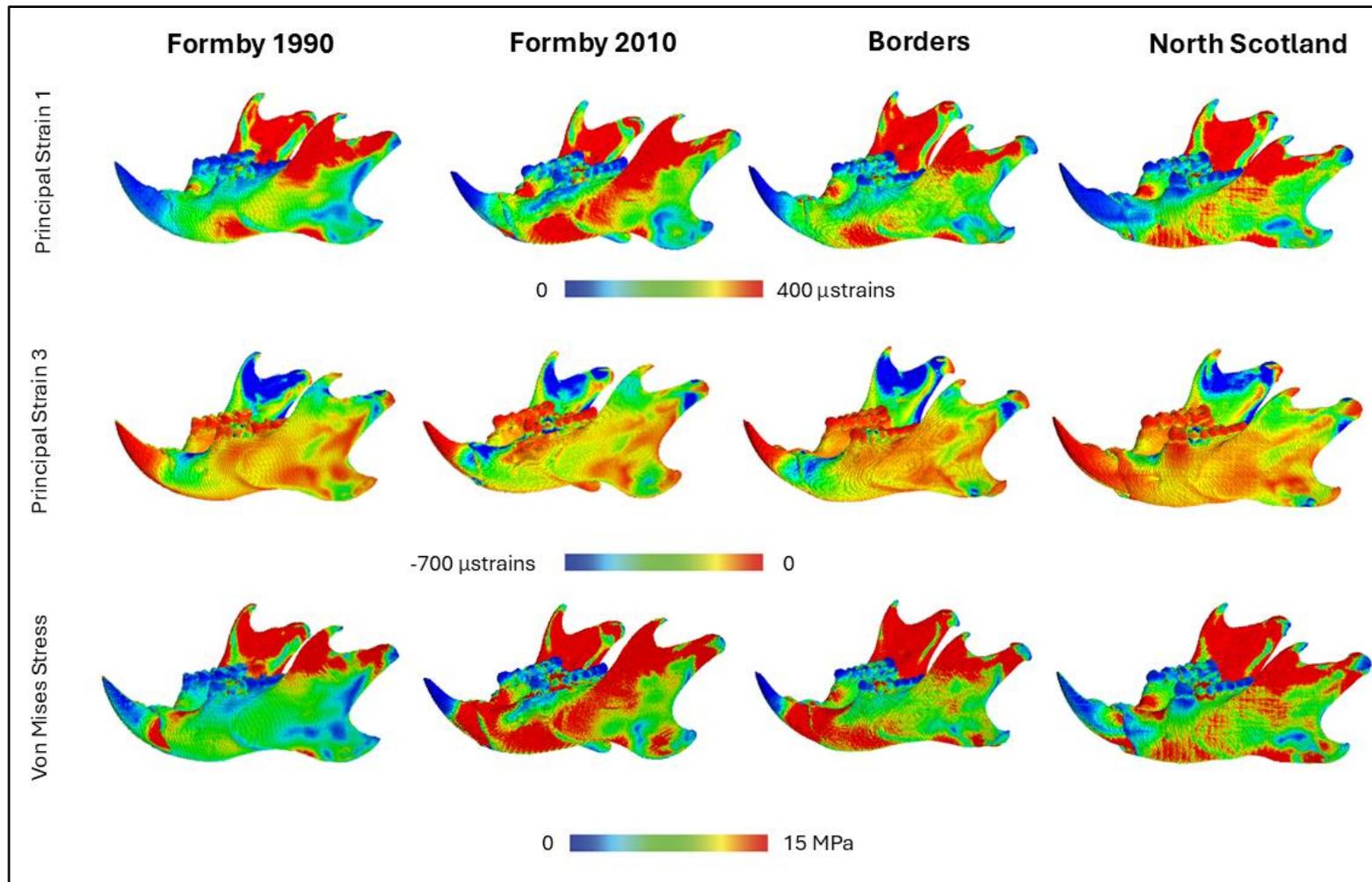


Figure 4.4. Colour maps of Principal Strain 1, Principal Strain 3 and von Mises stress across models of red squirrel mandibles under molar biting scenarios. Principal Strain 1 (tensile strain), Principal strain 3 (compressive strain) and Von Mises stress show highest strain in warm colours and lowest strain in cool colours. All colour maps were scaled to represent a maximum bite force of 20 N.

4.3.3 Mechanical Advantage

Overall, mechanical advantage did not vary hugely between models (see Table 4.7). Under incisor loading, Formby 2010s specimens had the highest mechanical advantage while the North Scottish specimens had the lowest. In molar loading, the trend was the same as incisor loading, with Formby 2010s having the highest mechanical advantage and North Scotland having the lowest.

Table 4.7. Values of bite force, as calculated through finite element analysis, and mechanical advantage calculated as ratio of input muscle force to bite force of mandibles of four red squirrels from four populations.

	Incisor Loaded		Molar Loaded	
	Bite Force (N)	Mechanical Advantage	Bite Force (N)	Mechanical Advantage
Formby 1990s	22.24	0.257	42.97	0.497
Formby 2010s	21.64	0.267	40.39	0.499
Borders	19.29	0.251	35.73	0.465
North Scotland	19.07	0.234	36.78	0.452

4.3.4 Virtual Ablation – Incisor Loading

Virtual ablation of the temporalis muscle of models under incisor loading reduced the median values of tensile strain, compressive strain and von Mises stress across all models (See Table 4.8). This is evident in heatmaps (see Figures 4.5, 4.6 and 4.7) which show a removal of areas of high stress and strain, predictably, in the coronoid process, across all models.

Mixed results were shown in the median values of stress and strain of incisor loaded models with virtual ablation of the superficial and deep masseters. Some models experienced a decrease in values compared to non-virtually ablated models and some showed an increase (See Table 4.8). Heatmaps do not show major differences in patterns of high stress and strain after virtual ablation of the superficial or deep masseters (See Figure 4.5, 4.6 and 4.7).

However, heatmaps of the Formby 1990s model with virtually ablated deep masseters, shows a concentration of high areas of tensile strain and von Mises stress in the coronoid process.

Table 4.8. Median Values for Principal Strain 1, Principal Strain 3 and Von Mises Stresses experienced and calculated mechanical advantage under incisor loading of virtually ablated models.

	Scaled	No Temporalis Muscle	No Superficial Masseter	No Deep Masseters
Principal Strain 1/Tensile Strain (μstrain)				
Formby 1990s	141.2	132.6	140.9	141.0
Formby 2010s	155.3	140.9	159.6	174.5
Borders	159.3	141.0	184.4	188.4
North Scotland	171.5	155.0	161.8	135.7
Principal Strain 3/Compressive Strain (μstrain)				
Formby 1990s	130.1	124.8	129.4	153.5
Formby 2010s	144.1	131.8	145.7	147.1
Borders	150.3	147.5	160.7	160.5
North Scotland	160.4	152.4	122.9	-
Von Mises Stress (MPa)				
Formby 1990s	6.2	6.1	6.2	6.9
Formby 2010s	6.5	6.2	6.6	7.1
Borders	7.0	6.8	7.6	8.0
North Scotland	7.1	6.7	6.7	6.0
Mechanical Advantage				
Formby 1990s	0.257	0.263	0.239	0.152
Formby 2010s	0.267	0.284	0.241	0.209
Borders	0.251	0.271	0.229	0.186
North Scotland	0.234	0.267	0.228	0.193

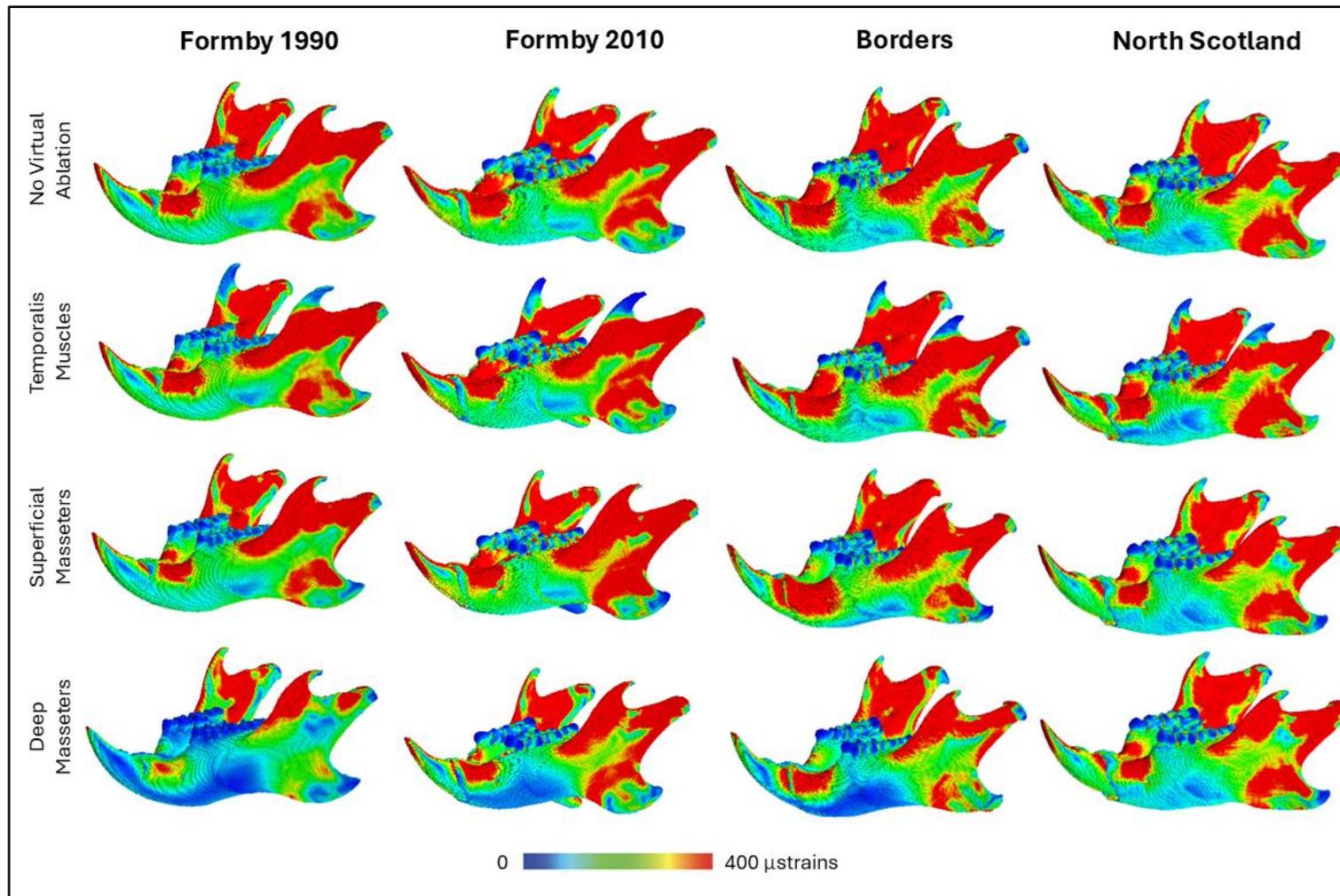


Figure 4.5. Colour maps of Principal Strain 1 in red squirrel mandibles under incisor biting scenarios, with virtual ablation of specific muscles. Highest strain is represented by warm colours and lowest strain by cool colours. All colour maps were scaled to represent a maximum bite force of 20 N.

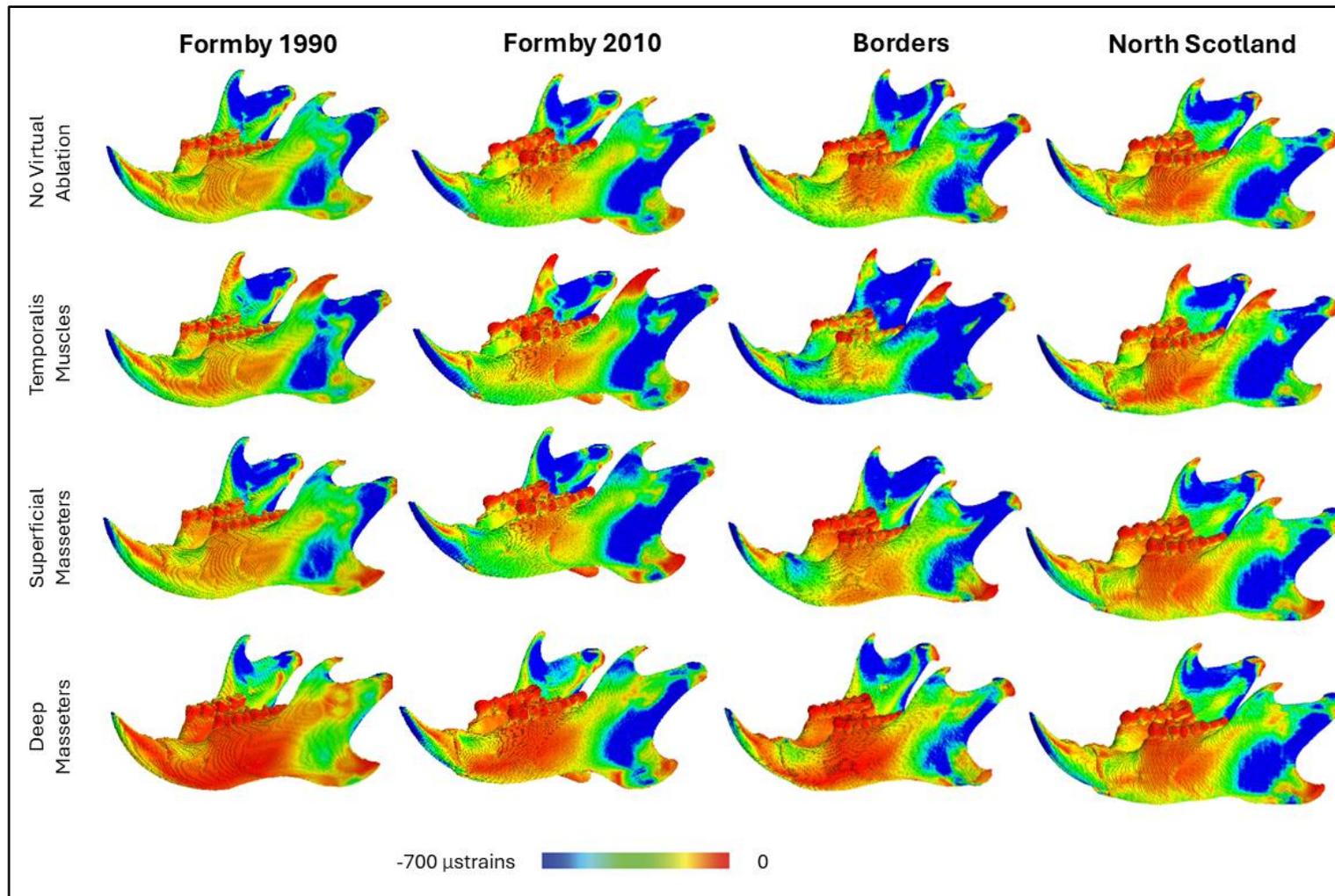


Figure 4.6. Colour maps of Principal Strain 3 in red squirrel mandibles under incisor biting scenarios, with virtual ablation of specific muscles. Highest strain is represented by warm colours and lowest strain by cool colours. All colour maps were scaled to represent a maximum bite force of 20 N.

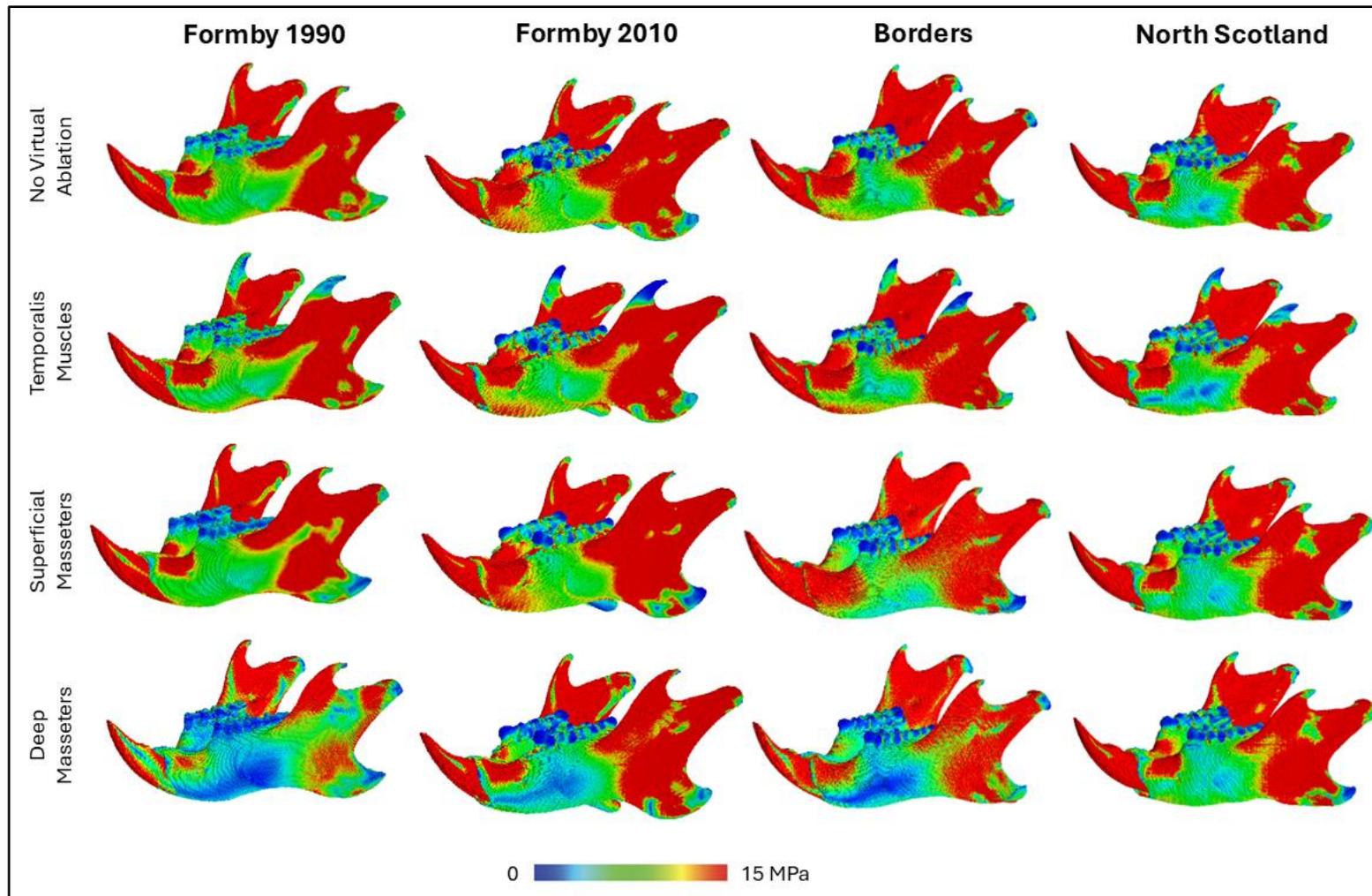


Figure 4.7. Colour maps of von Mises stress in red squirrel mandibles under incisor biting scenarios, with virtual ablation of specific muscles. Highest stress is represented by warm colours and lowest stress by cool colours. All colour maps were scaled to represent a maximum bite force of 20 N.

4.3.5 Virtual Ablation – Molar Loading

Under molar loading, virtual ablation of the temporalis muscle saw a reduction in the median stress and strain experienced by all models, while virtual ablation of the deep masseters saw an overall increase in median stress and strain. Virtual ablation of the superficial masseters saw mixed results (See Table 4.9). Heatmaps (see Figures 4.8, 4.9 and 4.10) revealed that under molar loading, removal of the temporalis muscle predictably saw a reduction in stress and strain on the coronoid in all models except in the Formby's 1990s model which showed a further spread of areas of high stress and strain across the ramus, and no evident reduction of stress and strain in the coronoid process. This is particularly evident in the heatmaps of von Mises stress (see Figure 4.10) .

Virtual ablation of the superficial masseters of models under molar loading gives mixed results in terms of increasing or decreasing the median stress and strain and heatmaps don't reveal major differences in stress and strain patterns. However virtual ablation of the deep masseters appears to overall increase the median stress and strain experienced by the models (see Table 4.9). Differences in patterns of stress and strain are not particularly evident in heatmaps (see Figures 4.8, 4.9 and 4.10). However as the main areas of stress and strain of models under molar loading are experienced in the coronoid process, a visible change after removal of the masseters is unlikely.

Table 4.9. Median Values for Principal Strain 1, Principal Strain 3 and Von Mises Stresses experienced and calculated mechanical advantage under molar loading of virtually ablated models.

	Scaled	No Temporalis Muscle	No Superficial Masseter	No Deep Masseters
Principal Strain 1/Tensile Strain (μstrain)				
Formby 1990s	113.9	104	105.4	128.0
Formby 2010s	130.3	122	135.4	140.5
Borders	134.4	124.4	140.1	146.9
North Scotland	121.6	121.1	120.1	152.
Principal Strain 3/Compressive Strain (μstrain)				
Formby 1990s	108.7	97.4	105.4	114.7
Formby 2010s	129.2	121.3	130.9	125.4
Borders	123.8	114.2	128.6	124.5
North Scotland	122.4	122.8	120.2	137.7
Von Mises Stress (MPa)				
Formby 1990s	4.9	4.9	4.8	5.7
Formby 2010s	5.8	5.6	5.9	6.0
Borders	5.7	5.4	6.0	6.4
North Scotland	5.6	5.7	5.5	6.7
Mechanical Advantage				
Formby 1990s	0.497	0.519	0.458	0.370
Formby 2010s	0.499	0.512	0.445	0.394
Borders	0.465	0.491	0.408	0.339
North Scotland	0.452	0.484	0.401	0.344

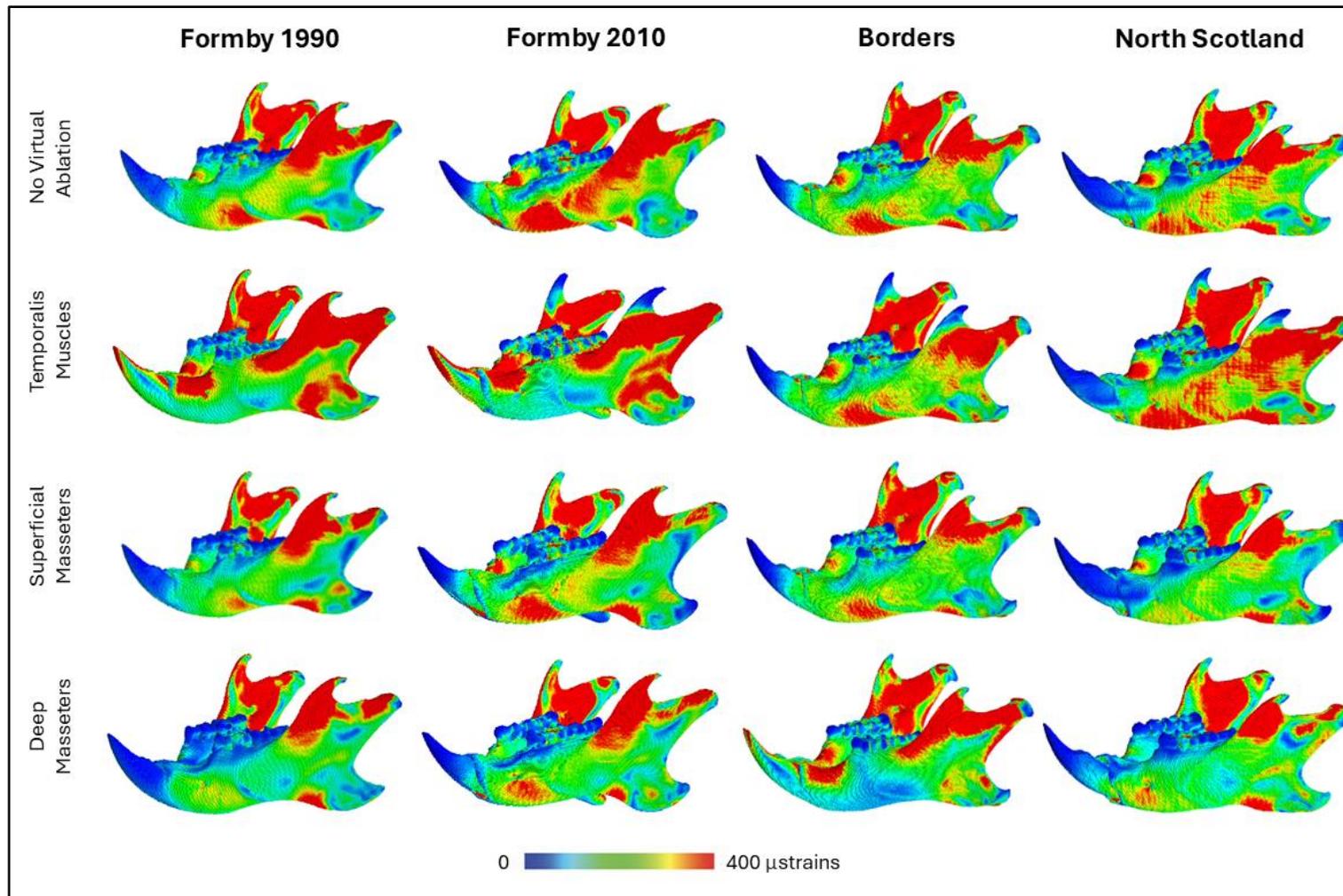


Figure 4.8. Colour maps of Principal Strain 1 red squirrel mandibles under molar biting scenarios, with virtual ablation of specific muscles. Highest strain is represented by warm colours and lowest strain by cool colours. All colour maps were scaled to represent a maximum bite force of 20 N.

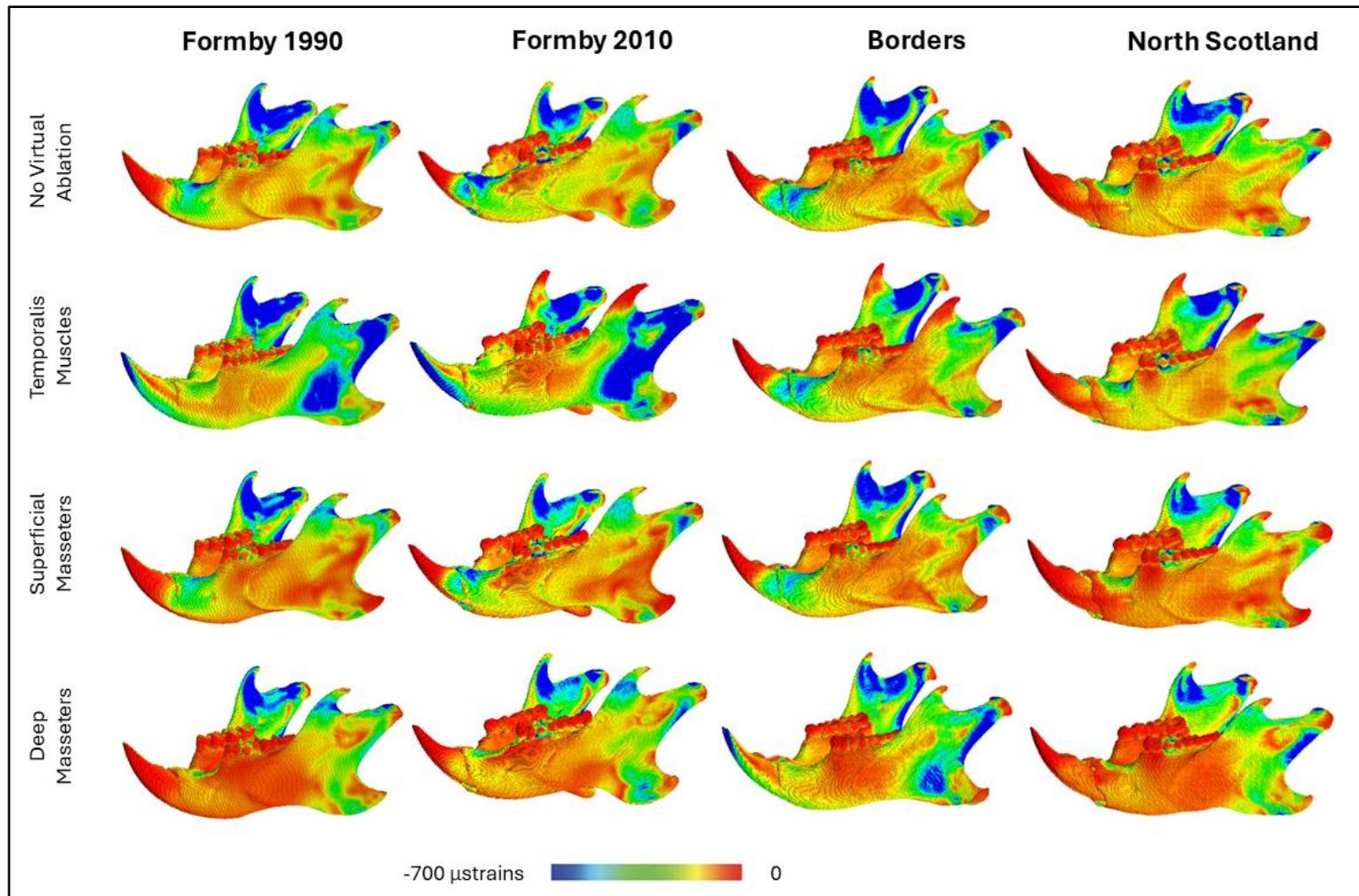


Figure 4.9. Colour maps of Principal Strain 1 red squirrel mandibles under molar biting scenarios, with virtual ablation of specific muscles. Highest strain is represented by warm colours and lowest strain by cool colours. All colour maps were scaled to represent a maximum bite force of 20 N.

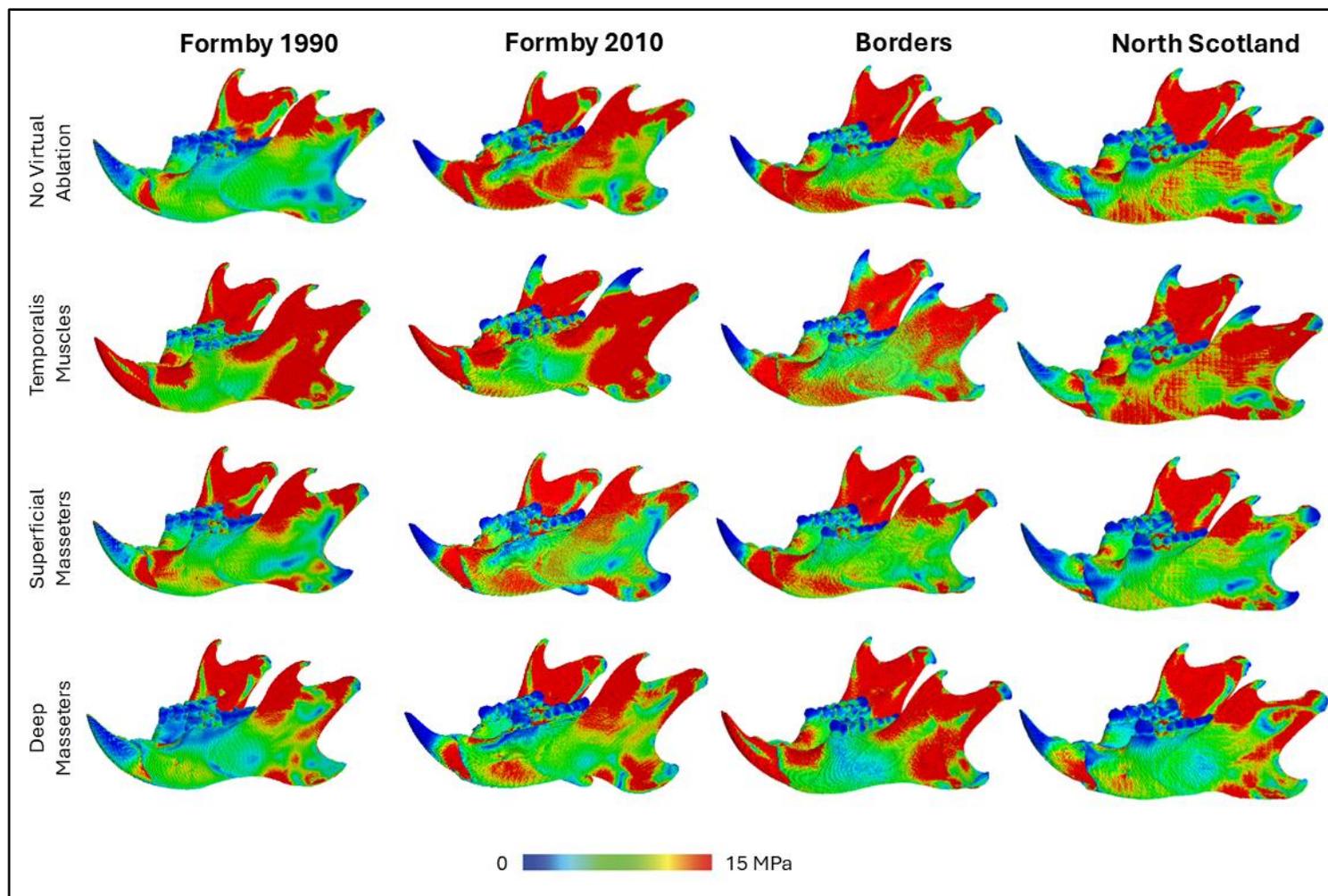


Figure 4.10. Colour maps of Principal Strain 1 red squirrel mandibles under molar biting scenarios, with virtual ablation of specific muscles. Highest stress is represented by warm colours and lowest stress by cool colours. All colour maps were scaled to represent a maximum bite force of 20 N.

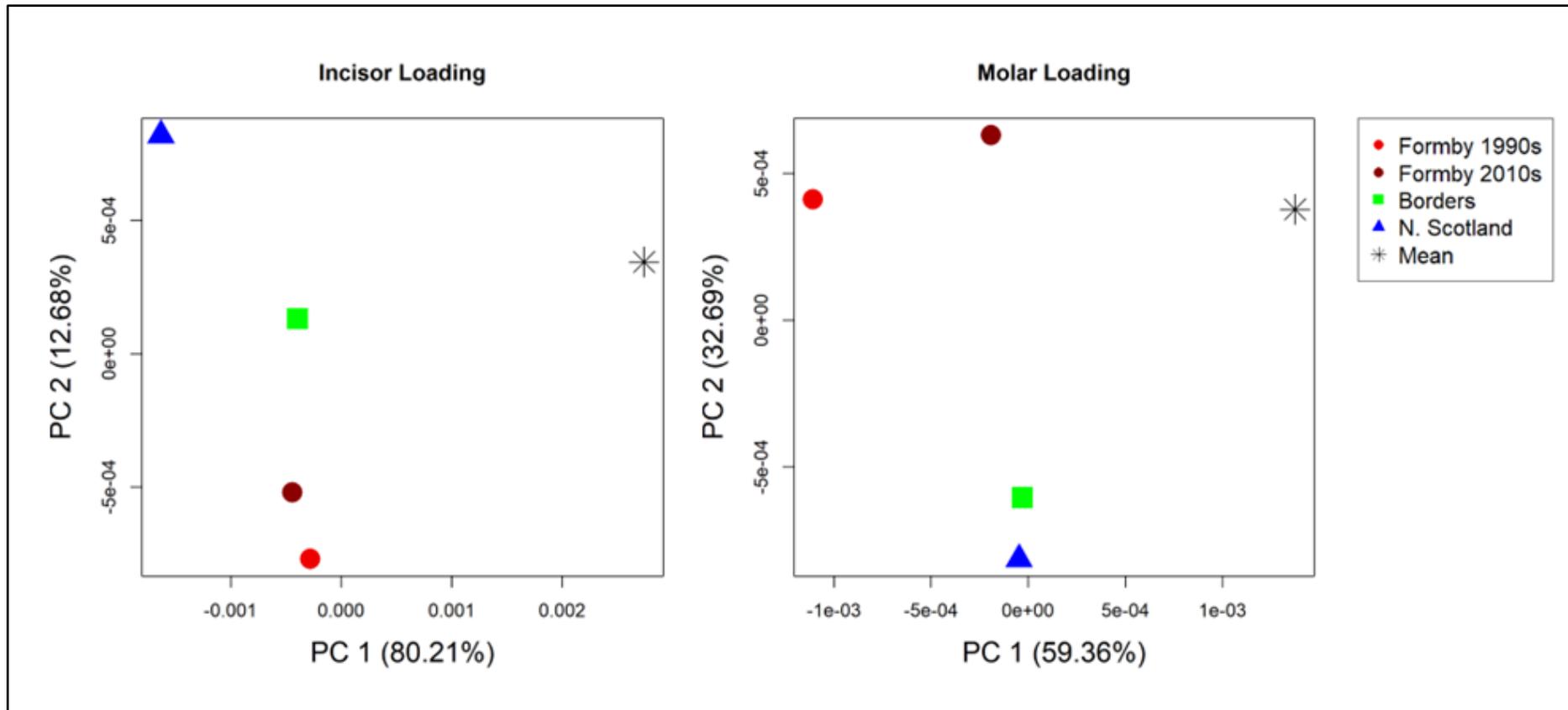


Figure 4.11. Principal component analysis (PCA) plot representing the differences of deformations between the four mandible models representing the four populations analysed, under both incisor and molar loading, as well as a mean unloaded model.

4.3.6 Geometric Morphometrics

Geometric morphometric analysis reveals that under incisor loading all models deform (change in shape from the mean model shape coordinates) in the same direction along PC 1 (responsible for 80.21% of shape change) but different directions along PC 2 (responsible for 12.68% shape change), with the two Formby populations deforming more similarly (in the negative values of PC2), than either the North Scottish or Borders model (see Figure 4.11). The North Scottish deforms the most from the mean model (see Table 4.10).

A similar pattern is seen in the geometric morphometric analysis of models under molar loading, which show that both the Formby models deform in the same direction along PC 1 (responsible for 59.36% of shape change) and PC 2 (responsible for 32.69% of shape change) although the Formby 1990 model deforms further along PC 2 and is overall the furthest in shape from the mean model shape (see Figure 4.11 and Table 4.10).

Table 4.10. Procrustes distance between models and the mean model, of models both under incisor loading and molar loading.

	Incisor Loaded	Molar Loaded
Formby 1990s	0.0032	0.0025
Formby 2010s	0.0033	0.0017
Borders	0.0033	0.0018
North Scotland	0.0044	0.0019

4.4 Discussion

4.4.1 Stress and Strain under Incisor Loading

Initial results suggest that the first hypothesis, a mandible model representative of the mean shape of the mandible of red squirrel specimens collected from Formby National Reserve in the 1990s would experience higher tensile strain, compressive strain and von Mises stress than model mandibles from three other populations, should be rejected. The model from the Formby 1990s population appeared to cope the best with the applied load, having the lowest median values for stress or strain. This was followed by the Formby 2010s model, then the Borders model and finally the North Scotland model experiencing the highest stress and strain. Median values are, however, only a general idea of the overall experience of the structure under loading, taken across the entirety of the mandible, including areas that are unlikely to experience stress or strain under loading. Therefore it is also important to assess the patterns of stress and strain across the structure. Results from the heatmaps are more nuanced. Largely, they also support the above conclusion that the Formby 1990s model experienced the lowest overall stress and strain. All models under incisor loading experienced the highest tensile strain in the coronoid and condylar region, as well as the angle and the diastema, but the area of low tensile strain on the ramus, between the angle and the coronoid-condyle region, was widest in the Formby 1990s model, suggesting the Formby 1990s model is coping best. However, the North Scottish model had the lowest values for tensile strain and in the body of the mandible, suggesting that the shorter, deeper mandible was better able to cope with stress and strain here than the more gracile mandible of the Formby 1990s model. The areas of high compressive strain on all models were concentrated in the region between the condyle and the angle, extending dorsally towards the body of the mandible, again to a lesser extent in the Formby 1990s model. High values of von Mises stress were experienced across most of the ramus and in the diastema across all models, with again the Formby 1990s model experiencing least and the North Scottish model have low values of stress in the body of the mandible.

Geometric morphometric analysis of deformation landmarks reveals differences in patterns of deformation under loading between the models analysed. By extracting landmark coordinates from deformed models, after loading, and comparing them to mean landmarks of non-deformed models it is revealed to what extent models experience change in shape under loading. The North Scottish model deforms to be most different in shape from the mean

model, which is consistent with the above findings that the North Scottish model experiences the greatest stress and strain under incisor loading.

4.4.2 Stress and Strain under Molar Loading

The results here support the second hypothesis, that when loaded as molar biting, the models will experience different levels of stress and strain. Under molar loading the models in general experienced less tensile strain, compressive strain and von Mises stress compared to incisor loading. This was not necessarily expected considering the rodents extreme evolutionary adaptations towards incisor biting. Again, the Formby 1990s model experienced the lowest median stress and strain values. Patterns of stress and strain visualised in heatmaps in Figure 4.4 show that under molar loading the areas of high stress and strain are localized in the coronoid process and along the lower edge of the mandible, with some high areas of tensile strain and von Mises stress also on the condyle. Unlike under incisor loading, there is little tensile strain or von Mises stress in the diastema. Compressive strain is mainly localised around the medial upper ramus and some in the diastema. This does not necessarily go against our assumptions that the diet of Formby squirrels would influence adaptations towards a softer diet. As the ‘softness’ or ‘hardness’ of the food items was focused primarily on the shell of the food items, which the incisors are used to fracture. The molars would be used mainly to process the edible kernels of the food items, which are generally similar levels of ‘softness’, although this was not assessed in Chapter 3. Potentially, the small size of pine seeds, reduces the need for molar mastication, therefore squirrels consuming a diet mostly of pine seeds (such as those in North Scotland) are not being selected for a strong molar bite.

Deformation of the models under molar loading was also assessed through geometric morphometrics. Results indicated that despite performing relatively well under molar loading, as evidenced by the median values of stress and strain and in patterns of stress and strain in the heatmaps, the Formby 1990s model deforms the most from the mean model. This is surprising, but is likely due to a high level of deformation occurring in one small portion of the mandible, mostly likely the coronoid process.

4.4.3 Mechanical Advantage and Bite Force

The third hypothesis, that mechanical advantage of the Formby 1990s model mandible will be lower than that of other populations, is rejected. As previously discussed, the relative softness of the diet of Formby 1990s specimens is thought to have removed the selection pressure towards high bite forces, while the relative hardness of the diet of Borders and North Scottish specimens is likely to drive a change towards high bite forces. Mechanical advantage (hereafter occasionally referred to as MA) is a measure of the proportion of muscle force translated into bite force. Therefore, bite forces, as calculated through FEA, are a predictor of mechanical advantage. Assessing the ratio of muscle force to bite force removes any differences in force generated due to different mandible sizes. However, even with these caveats, the results for mechanical advantage still subvert our expectations, with the North Scottish model having the lowest mechanical advantage and the Formby 1990s model having the highest, under incisor loading. Similarly, under molar loading, the Formby models both have higher MA than the North Scottish and Borders model. However, here, there was virtually no difference in MA between Formby populations, suggesting that the morphological variation observed in Chapter 2 have more effect on incisor biting efficiency than molar biting efficiency.

The use of this method of analysing mechanical advantage is interesting as we can compare and contrast with methods used in Chapter 2. In Chapter 2 the mechanical advantage was calculated from linear measurements of the jaw, taken from photographs, and calculated as the ratio of the muscle in-lever to the biting out-lever (as described in Casanovas-Vilar & van Dam, 2013; Cox et al., 2020). Two muscles were analysed, the dorsally inserting fibres and ventrally inserting fibres of the temporalis muscle, both as if in incisor biting. Both these muscle fibres showed the Formby 1990s specimen to have the lowest mechanical advantage compared to the other populations analysed, and a Pearson's product-moment correlation coefficient test showed that this was not related to size but to overall shape. Mechanical advantage calculated from linear measurements, is relevant to the specific muscle of which the in-lever is measured. However, in calculating MA from results of FE models, we can calculate MA as the ratio of output bite force to input muscle force. This uses muscles forces for the entire masticatory apparatus, rather than looking at one specific muscle. Therefore, having differing results from the two methods is not impossible. The mechanical advantage of the temporalis muscle could be lower in a mandible but the MA of the system overall

could be higher. This appears to be the case in the Formby model. The morphology of the mandible, a shortened distance between the coronoid and condyle, results in a shorter muscle in-lever, lowering the MA of the temporalis muscle (when calculated through linear measurements). However it appears that the MA of other muscles in the system are increased, causing an overall increase in MA (as results show from MA calculated from FE models). It appears that although the shortened temporalis muscle in-lever in the Formby 1990s specimen should have negative consequences for the MA of the system as a whole, the rest of the system is compensating in some capacity.

4.4.4 Impact of Musculature

The fourth hypothesis, that the virtual ablation of the masseter and temporalis muscle will remove most differences in stress and strain between specimens is also unsupported, with varying effects on the difference between stress and strain values between models. However the removal of muscles does reveal insights into the functioning of the muscles of mastication in both incisor and molar loading.

The reduction in median strain and stress experienced with removal of the temporalis muscle is largely due to reduction in the overall strain and stress applied to the coronoid process. The temporalis muscle is heavily used in incisor biting so the diversion of the relative muscle forces of the temporalis muscles to the other muscles of mastication has a predictable reduction in overall stress and strain experienced in the coronoid. We see this removal of stress and strain on the coronoid process in the heatmaps in (Figure 4.5, 4.6 and 4.7). The slightly lesser reduction in median stress and strain across all models under molar loading suggests that the temporalis has a slightly lesser role in molar chewing than incisor biting. However, the results of the mechanical advantage suggest otherwise. Virtual ablation of the temporalis muscle in models under incisor loading caused an increase in mechanical advantage, indicating that higher muscle forces in the other muscles of mastication (the masseters, the medial and lateral pterygoids and the zygomaticomandibularis) is more beneficial in increasing the bite force. This was also the case of in molar loading where removal of the temporalis muscle also raised mechanical advantage across all models.

Under molar loading, the removal of the superficial masseter and deep masters had mixed results on the models. The Borders and Formby 2010s models experienced increased overall

stress and strain with removal of both the superficial masseters and the deep masseters. As the relative muscle forces are then allocated to other muscles of mastication, this could be due to increased strain and stress applied to the coronoid process by the temporalis muscle. In all models except the Formby 1990s the heatmaps do not reveal significant differences in patterns of tensile strain, compressive strain or von Mises stress between the original models and models with virtual ablation of the masseters. The Formby 1990s model shows a reduction of median stress and strain across with the removal of the masseters. This, supported by patterns of strain and stress distribution suggests that the Formby 1990s model is better able to cope with increased force applied through the temporalis muscle.

4.4.5 Conclusions and Further Work

Overall the results of this study have created a puzzling picture of the biomechanical performance of the red squirrel mandible in British populations. The goal of this study was to elucidate the biomechanical effects of the specific morphological differences made clear in Chapter 2. Which, despite unexpected results, has been achieved. Above we see that the distinctive curvature of the coronoid process in the Formby model appears to have the greatest effect on the temporalis muscle. Shortening the temporalis in-lever decreases the MA of the temporalis muscles in Chapter 2, however results here indicate that the overall MA does not suffer from this reduction. The Formby 1990s specimen appears to cope best with stress and strain applied by the temporalis muscle compared to other models. Additionally, this muscle appears to affect the incisor biting more than in molar biting, with differences between the Formby 1990s model and the 2010 model (which has a coronoid process that is reduced in size). So overall, it appears that the temporalis muscle in the Formby model, does have an effect on function, but features of the other muscles of mastication, potentially supported by shape changes in the rest of the mandible negate any adverse effects. The North Scottish model, although appearing to perform worst under load, appears to prioritise resistance to stress and strain in the body of the mandible.

Due to time constraints, this chapter focuses solely on the mandible. As the mandible has one primary function, mastication, shape changes are likely to be driven by selection pressures associated with the diet. However, the mandible is not solely responsible for mastication. Chapter 2 also revealed shape variation in the cranium, indicative of different biomechanical abilities. All muscles of mastication have attachment sites both on the cranium and the

mandible, and differences in the shape of the crania could affect overall function of the muscles of mastication. Analysing the mandible solely does not reveal the whole picture of biomechanical performance of each individual. Further FE analysis of the mean cranial shape may reveal further differences in biomechanical performance between these populations.

Additionally, it must be conceded that the models included in this study are only representative of the populations analysed as a whole. The geometric morphometric study in Chapter 2 is a powerful analysis in that it includes a large sample size of many specimens from each population whereas, this study has a single specimen representative of a whole population. The specimens were chosen carefully, each being a specimen closest to that of the mean shape of the population as a whole. Analysing multiple specimens from the same population may have led to results closer to what we initially expected, particularly if enough specimens were included to gain statistically significant results. However time and financial constraints of CT scanning and model building many specimens unfortunately prevents this from being a reasonable option within the scope of this study.

Finally, there are multiple necessary assumptions made in the creation of FE models. There are other adaptations that may have arisen in the mandible that the FE models cannot effectively model. For example, muscle force can be increased, not just by covering a larger surface area of bone, but packing more muscle fibre into an area (Bates & Falkingham, 2018; Broyde et al., 2021). Muscle fibre orientations can change to increase force (Broyde et al., 2021). Shorter muscle fibres can also increase the MA by increasing muscle force (Broyde et al., 2021). All features that are difficult to assess when working with skeletal material only. A method to deal with this would be to perform dissections on specimens representing each of these populations. Then muscles could be weighed and analysed for each model, and biologically accurate muscle forces applied to the models. Additionally, this study was limited to investigating how variation of shape of the mandible affects biomechanical function, however there are other factors that affect feeding biomechanics. One of these is size. As muscle forces were scaled in this study, most of the differences in biomechanical performance due to size were removed (although some allometric component to mandibular shape will remain). A larger squirrel may have a morphology that is less efficient or less well optimised to biting at high forces, however the larger size of the muscles will allow for generation of higher bite force. In conclusion, while the results of the FE study go some way into assessing how well different structures perform biomechanically, the necessary

assumptions that are made in model creation, may be leaving out vital information that also contributes to the overall functioning of the system.

5. Discussion

This thesis has achieved its aim of analysing an interesting case of morpho-functional divergence within a population of animals experiencing fragmentation through human-mediated ecological change. The morpho-functional divergence in the masticatory apparatus of British red squirrels has been analysed in a three-pronged approach of assessing variation in morphology, function and the potential driver of morphological change, diet.

5.1. Assessing Morphological Variation, Spatially and Temporally, in British Red Squirrel Populations

Chapter 2 aimed to assess whether it was possible to detect morphological variation in the mandible and crania of British red squirrels, both between populations and over time. Through study use of an excellent collection of red squirrel skeletal specimens at the National Museum of Scotland, photographing and 3D scanning specimens, and assessing the morphology through geometric morphometrics, this aim was achieved. A significant difference was detected between three populations in cranial morphology and between four populations in mandibular morphology. Temporally, it was detected that there was a significant difference in shape within Formby populations, with those specimens collected before 2000 and those collected after 2010 having statistically significant mandibular and cranial shape. This adds further support for research published by Cox et al. (2020), supporting their conclusions and expanding insight into cranial morphology and temporal change.

The morphology of the different populations allows conclusions regarding potential functional impacts to mastication. The North Scottish and Borders specimens show adaptations towards higher bite forces (e.g. larger temporalis muscles, robust mandibles). While the morphology of the Formby population suggests a lesser bite force (e.g. more gracile mandibles, smaller temporalis muscles). Conclusions on functional consequences of the morphological variation are speculative however, necessitating further assessment of biomechanical performance (later explored in Chapter 4). Despite this, simple linear measurements could be taken in order to gain estimates for mechanical advantage of the mandible, allowing preliminary conclusions on the mechanical efficiency of the mandible. Results of these estimates indicate that there is a link between the morphology and function, with the Formby specimens showing overall lower values for mechanical advantage and the North Scottish and Borders specimens showing higher values.

The Formby population shows distinctiveness in both its unique morphology, and morphological change over time. Formby specimens are generally larger than most other specimens (except Jersey) and have a distinctly curved coronoid process. However, when separated into two sub populations (1990s and 2010s), the Formby 2010s specimens have less pronounced curvature of the coronoid process and are reduced in size.

When searching for an explanation for the morphological differences between populations, the most parsimonious conclusion would be an association with diet. As discussed in Chapter 1, the mandible is highly adaptable and with mastication as its sole evolutionary purpose, it is fair to conclude that any changes in morphology is likely related to changes in food consumption. When we look at the diet of the populations analysed we see distinct differences related to habitat. Pine forests in North Scotland provide pine seed as a main source of calories, deciduous forests offer hazelnuts, acorns, beech seeds and sweet chestnuts in Northern England and Jersey. Formby is interesting in that the population here has been heavily supported by conservationists who, in the past, provided supplementary food in the form of peanuts to squirrels in Formby.

5.2 Investigation of the Material Properties of Red Squirrel Food items and the effect of caching.

In Chapter 3, the potential link of morphology to diet was investigated. The aim of this chapter was to create a profile of the mechanical challenges of the differing diets to the four populations of British Red Squirrels analysed in Chapter 2. To achieve this aim, a method of gathering data on the toughness (mJ/mm), force to fracture (N) and energy to fracture (mJ) of the external shell of seven food items was devised. The method of gathering toughness estimates proved useful in facilitating comparisons between food items such as larger nuts with hard shells (e.g. hazelnuts) and smaller food items without hard shells (e.g. pine seeds which are encased in cones). The results of the study confirmed that peanuts are significantly easier for squirrels open than other food items. As peanuts made up a large portion of the diet consumed by squirrels in Formby in the 1990s, this supports conclusions above, that the relative ‘softness’ of the diet of one subpopulation has encouraged an adaptive change in the masticatory apparatus of that subpopulation.

Low toughness values for pine cones proved interesting, as this demonstrates the need to not only look at the material properties of the food items, but also consider the method in which the animals consume the food item. Although pine seeds appear relatively easy to access - in

terms of creating the necessary bite force to access the edible seeds - the small size of the food item, and the method of accessing (removing the scales from the cones) requires a frequently repeated action to gain sufficient nutritional value from the food item, putting a particular pressure on the animal that we cannot disregard.

This study also, in an attempt to assess the mechanical challenges of food items authentically, looked at whether a frequently performed behaviour, caching, affects the ‘hardness’ or ‘softness’ of the food items. Caching, as well as being a method of ensuring food supply in months where food is scarce, may have some benefits in making food items easier to masticate. However, results of caching experiments in Chapter 3 do not reveal any consistent trends, with mixed results on whether caching increased or decreased the relative toughness of or force/energy required to access food items. Regardless, the results of this study support the overall hypothesis that commonly consumed food items in some populations would be overall more difficult to access mechanically than others. It is the Borders populations, which has wider access to the most difficult to access food items (a mix of nuts from deciduous trees and pine seed), that has the highest selection pressure towards high bite forces while Formby 1990s likely has the lowest.

The tentative conclusions of Chapter 2 are also supported, in that the change in morphology of the Formby’s specimens between 1990 and 2020 is likely linked to change in diet. In the early 2000’s (Shuttleworth, 2000) conversationists at Formby decided to stop the provision of such a high quantity of peanuts to the squirrels. This rapid reduction necessitated a return to the consumption of pine seeds, a tougher food item. The morphology of the squirrels at Formby post 2010 is indicative of a return to the need for a higher bite force. In particular the loss of the highly curved coronoid process, reduces the length of the muscle in-lever, therefore increasing mechanical advantage.

5.3 Investigation into Biomechanical Function of the Red Squirrel Mandible using Finite Element Analysis

Chapter 2 and 3 investigated the morphological variation of the red squirrel masticatory apparatus across the three main populations, gaining insights into potential functional effects of morphology, and established likely drivers of this morphological change (diet). The final piece of the picture is to link those morphological traits to any functional adaptations towards diet. In Chapter 4, finite element analysis is employed to assess the biomechanical performance of mandibles of four sub-populations – North Scotland, Borders and two from

Formby, one from the 1990s and one from the 2010s. As previously discussed, it was expected that the morphology of the Formby 1990s specimen, consuming a ‘softer’ diet and having traits indicative of a lower bite force, would perform worse than other mandibles, experiencing high values of stress and strain across the mandible and have an overall lower bite force. Interestingly, the results of FEA show a more complicated picture.

For median values of tensile strain, compressive strain and von Mises stress across the entire mandible, the Formby 1990s model had the lowest values, under both incisor and molar loading, while the North Scotland model had the highest, the opposite to what we might expect. However, as discussed in Chapter 3, median stress and strain across the entire structure is not particularly representative of how the structure is performing overall. Looking at the heatmaps gives a clearer picture of how the models are behaving. Here the picture is complicated. Although some observations support the conclusions above (that the Formby 1990s model performed best under load, while the North Scottish model performed worst), there are additional nuanced observations to be made. Under incisor loading, tensile strain was mostly concentrated around the coronoid and condylar processes, an expected result considering the placement of the masticatory muscles. Areas of low tensile strain were widest on the ramus of the Formby 1990s model, suggesting an overall better performance.

However, in the North Scottish model, the body of the mandible shows very low values of tensile strain. Suggesting that, although the Formby 1990s mandible experiences the lowest strain and stress overall, the morphology of the North Scottish model allows for robustness in the mandible body. Results from Chapter 2 showed that generally the mandible of squirrels from North Scotland were deeper, shorter and had a less well-developed coronoid process. The deeper, shorter, body of the mandible may help reduce the amount of strain experienced in this area.

Results from Chapter 3 demonstrated that the diet of the Borders population poses the hardest mechanical challenge to squirrels. Therefore, we expect this model to perform best under stress and strain. However, this model appeared to perform somewhere middling compared to other models. Deciduous tree seeds (hazelnuts, acorns) were the most difficult to access, with mean force requirements between 164.82 – 203.29 N to fracture the outer shell. The bite force estimates from the Border model suggest a highest bite force of only 19.29 N at the incisors or 35.73 N at the second molar. Of course, the force to fracture estimates from Chapter 2 are purely for comparison between food items, where a flat plate was applied to the whole shell. It is unlikely that the squirrels are applying pure compressive load onto the food

items, employing more complicated masticatory movements to access the food items, such as gnawing. Incisor teeth also allow all energy and force to be applied to a specific point, so the amount of force needed to be generated will be lower. Despite this, we would still expect the Borders population to be able to generate higher bite forces than other models.

Also discussed in chapter 4, are other adaptations that may have arisen in the mandible that the FE models cannot effectively model. Features such as having more muscle fibre packed into an area, muscle orientations changing in a way that increases the force or shorter muscle fibres could all increase overall bite force without leaving much evidence on the mandible bone. The only way to assess whether this is occurring is through dissection and observing the muscles of specimens from the main populations.

Despite conflicting results regarding what we tentatively conclude about function from the morphology, and the biomechanical performance concluded from FE studies, some interesting insights are made. The Formby specimens, despite having morphology suggestive of a weaker bite, appears to have adapted in other aspects of its anatomy to compensate for the potential reduction in mechanical efficiency of the mandible. Squirrels from North Scotland have prioritised resistance to stress and strain in the body of the mandible rather in the ramus. Potentially these traits are beneficial in conferring selective advantages to functions that are not modelled here. For example, we know that the method of accessing pine seeds is relatively different to the method in which nuts are fractured. The cone scales are removed methodically, through biting through a scale (having relatively low toughness as demonstrated in Chapter 3) and pulled away to reveal the pine seed. As such the mandible may not need to be able to withstand stress and strain in the ramus, when incisor biting, but instead withstand stress and strain in the body in the ‘gripping and pulling’ motion required to remove the scale.

5.4 Limitations

5.4.1 Other drivers of morphological change

There are other potential reasons where morpho-functional variation may occur. When looking at small, isolated populations, the possible effects of inbreeding cannot be ignored. The Formby population is relatively small, isolated to the woodland in which they were introduced from Europe in the 1930s (tentatively stated in Gurnell & Pepper (1993)). The details of the introduction are not recorded so whether the initial introductory population was only a few individuals is unknown. If so, this population likely experience the founder effect.

Furthermore, the Formby population has suffered a number of population crashes due to outbreaks of squirrelpox, the most disastrous of which was in 2008 (Chantry et al., 2014) which reduced the population by 87%. Population crashes contribute to the likelihood of genetic bottlenecks arising which may influence morphological traits through genetic drift. Reduction in population size then may allow inbreeding to occur. As discussed in Cox et al. (2020), some authors have suggested negative effects on the biomechanical abilities of the rodent mandible related to inbreeding (Leamy et al., 2002). Other studies, in contrast, found no relationship (Markow, 1995) with one study finding no impact of inbreeding depression on bite force in a study on inbred and outbred mice (Ginot et al., 2018).

In efforts to determine whether the diet of Formby red squirrels was indeed significantly different to other populations, research into isotopic ratios of the bone collagen of red squirrel specimens was performed by collaborators at the University of Exeter and University of Reading (Chandler et al., 2024). Bone collagen from ribs of specimens used in this study was extracted and isotope ratios determined using mass spectrometry. The Formby 1990s population had the lowest overall mean $\delta^{15}\text{N}$ value, followed by Formby 2010s. Borders and Jersey populations had the highest. Peanuts, unlike other food items analysed in this study, are legumes and have a lower nitrogen isotope ratio than non-leguminous plants (Noe-Nygaard et al., 2005). This supports the assumption that the diet of the Formby 1990s population is significantly different from that of other British populations. There is a undeniable change in the diet over time. So regardless of whether the morphological change has been facilitated through genetic drift or inbreeding, the morphological traits that have persisted are very likely linked to the overall diet.

5.5 Interesting Revelations

5.5.1 Adaptive Plasticity vs Evolution

In Cox 2020, it was concluded that the morpho-functional distinctiveness of the Formby population was either an evolutionary response (similar to examples in: Cornette et al., 2012; Herrel et al., 2008; Renaud et al., 2015) or a plastic responsive (similar to examples in: Casanovas-Vilar & van Dam, 2013; Doudna & Danielson, 2015; West & King, 2018). Results of this thesis support the occurrence of an adaptive response. The temporal morphological change within the Formby population between the 1990s and 2010s occurred too rapidly for sufficient generation time for an evolutionary response to occur. This is

backed by the existence of a likely driver of masticatory adaptation (a change in diet), through isotopic analysis (Chandler et al., 2024).

Adaptive plasticity of the mandible towards dietary differences would be best assessed through ontogenetic studies. Ontogenetic studies have been used to confirm the mandibles ability to change in shape in response to mechanical needs through bone modelling (Anderson et al., 2014; Myers et al., 1996; Renaud & Auffray, 2010). Generally studies have taken place in a laboratory setting with mice, which is unsuitable for the study of red squirrels. However, if juvenile specimens were collected from various British populations and compared morphologically it could yield interesting results. The hypothesis would be that the mandible of juvenile specimens across populations would be morphologically analogous, while the adults would be morphologically diverse. This would definitively confirm that the morphological diversity is a result of adaptive plasticity.

Although it is important to note that evolution and plasticity are not entirely separate from each other, as plasticity may lead to heritable change over time through genetic assimilation (Badyaev, 2005; Parsons & Robinson, 2006). So although this population is adapting *in vivo* to a ecological pressure, this change may become heritable over time.

5.6 Further Work

5.6.1 Island Populations of Red Squirrels in Britain

Throughout much of this thesis I have reiterated the similarity of the fragmented, isolated populations of red squirrels in Britain as similar to those populations that exist on islands. Mayr (1963) considered islands to be natural laboratories for evolution, evidenced by the many cases of island rodent populations, where interesting phenomena, such as accelerated evolution, has been documented to occur (Abdelkrim et al., 2005; Doudna & Danielson, 2015; Renaud et al., 2015; West & King, 2018). Additionally, microevolution has been shown to be greater on the smaller and more remote islands (O. R. W. Pergams & Ashley, 2001). Three populations of Red squirrels in Britain that were not heavily scrutinised in this thesis were island populations – Isle of Wight, Brownsea Island and Anglesey. The reason for not including these populations in analyses was an insufficient number of individuals in the collection at National Museum of Scotland. This is unfortunate as the conclusions regarding their morphology and functional abilities would be interesting. In the Isle of Wight, they inhabit mixed woodland and suburban areas with plenty of access to supplementary food in gardens, allowing potential conclusions on morphology of red squirrels in semi-urban

environments. The Anglesey population are an introduced population with access to supplementary food (Shuttleworth, 2015) and the Brownsea Island population is small with limited genetic diversity (Hardouin et al., 2019; Thain & Hodder, 2015). All interesting features to analyse in terms of their morphological traits. These populations have a better chance of protection from encroaching grey squirrel populations through physical barriers (except perhaps Anglesey, which does have a connection to the mainland over which grey squirrels have travelled (Shuttleworth et al., 2015). As such, the potential for these populations to remain as safeholds for native British red squirrel populations is high and therefore worth continual examination.

5.6.2 Mainland Europe Populations of Red Squirrels

It would also be interesting to assess the morpho-functional traits of British red squirrels in the context of wider populations of red squirrels. In mainland Europe and parts of Asia, red squirrels are not an endangered species, listed by the IUCN as of least concern (Gazzard, 2023). Removal of the isolation experienced by the fragmented populations on mainland Britain allows for frequent genetic dispersal and morphological adaptations are likely slower to arise (Millien, 2006). However, as we have confirmed that the morphological adaptations seen in British red squirrels, particularly in Formby, are more likely a product of adaptive plasticity rather than micro-evolution, the morphological adaptation of the masticatory apparatus may also be occurring in specific habitat types. Additionally, Hale et al. (2004) suggested that the increase in a particular haplotype now commonly present in red squirrels in Northern England, likely dispersed from squirrels introduced from Scandinavia, may have some kind of competitive advantage in the spruce plantations of the Kielder forest, similar to what is available in Northern Europe. As such it would be interesting to study both the morphology and genetics of particular populations of squirrels in habitats similar to those in Britain.

5.6.3 Grey squirrels (Sciurus vulgaris)

The reasons for the grey squirrels ability to outcompete red squirrels in Britain was much investigated, with reasons such as interspecific aggression (Wauters & Gurnell, 1999), habitat (Gurnell et al., 2004b; Kenward et al., 1998) and diet (Kenward & Holm, 1993) explored, before squirrelpox was implicated as the main cause (Atkin et al., 2010; Bruemmer et al., 2010; Chantry et al., 2014; Parrott et al., 2009; Rushton et al., 2000; Tompkins et al., 2002). However, grey squirrels do still appear to be better generalists than red squirrels and thus

better competitors in areas experiencing habitat change (Kenward & Holm, 1993; Kenward & Tonkin, 1986) - through urbanisation, introduction of non-native timber plantations or deforestation, for example. Investigating the masticatory apparatus and diet of grey squirrels may reveal further differences in the ability of grey squirrels to thrive in many habitat types, an important goal in the further management of control of this invasive species.

5.7 Concluding Remarks

5.7.1 Supplementary Feeding as Conservation Tactic

In Gurnell and Pepper (1993) it was noted that the high density of Formby Red squirrels was considerably higher than what had usually occurred in vertebrate populations when provided food supplementation (Boutin, 1990). Gurnell and Pepper (1993) suggested it was necessary therefore to further investigate the Formby population to gain a better understanding of supplementary feeding as a conservation tactic. In this thesis we have further investigated supplementary feeding in the Formby population. It could be argued that the provision of supplementary food at Formby boosted the population to artificially high numbers, unsustainable in the natural habitat. However, after the population crash in 2008, the Formby population had almost reached pre-crash numbers (Miller, 2015). This is after the removal of peanuts as a supplementary food, so unlikely that the removal of this food source had significantly effected population numbers. Regardless, the provision of food at feeders or in specific locations encourages the gathering of animals and may facilitate the spread of disease (Hines et al., 2007; Sorensen et al., 2014; Tryland et al., 2019). Therefore, the choice of reduction of supply of supplementary food by conservationists at Formby was astute. If conservationists wish to continue to provide supplementary food as a buffer against fluctuations in the number of red squirrels, the methods in which the food items are provided. Additionally, as evidenced through morphological studies here, choose appropriate food items so as not to encourage the appearance of adaptations towards specific food items, whether through adaptive plasticity, micro-evolution or behavioural changes.

5.7.2 Final Remarks

In this thesis, the masticatory morphology of four major populations of red squirrels in Britian has been assessed, furthering knowledge of the acquired adaptations of a fragmented population. The diet of these fragmented populations has also been assessed in terms of their material properties and the mechanical challenges they pose to the squirrel populations, something not yet previously considered. And, finally, the biomechanical function of the

masticatory apparatus has been assessed, demonstrating the difficulty of comparing function interspecifically, with such minute differences needing to be assessed with a more holistic approach.

Through this, this thesis has shined a light on a previously unconsidered effect of fragmentation, isolation and reduction in numbers of a native species. The rapid expansion of grey squirrels in Britain provides an interesting case study in the devastating consequences that the introduction of a non-native species can have on native fauna. Unfortunately, grey squirrels *Sciurus vulgaris* are also present on mainland Europe, introduced to Italy in 1948 (Currado & Scaramouino, 1989; Currado et al., 1987). Although surviving comfortably, red squirrels here seem to not have suffered significantly due to the presence of grey squirrels, as these squirrels appear to not be carriers of SQPV. If they *were* to be infected however, the repercussions could be disastrous, resulting in a distribution similar to what we see in Britain today. Understanding how populations of red squirrels in Britain have been affected can help us understand future possible patterns of change.

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Appendix 1. Table of metadata related to red squirrel specimens used for analysis in Chapter 2.

Specimen Number	Location recorded on label	Population Group	Year Collected	Decade Collected	Sex	Parts Suitable for Landmarking
NS.1948.042	Penicuik	Borders	1948	pre 1960	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch
NS.1961.023	Penicuik side of Peebles	Borders	1961	1960	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch
NS.1966.020.008	Pitlochry	North Scotland	1957	pre 1960	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch
NS.1990.024.004	Tyringhame Woods East Lothian	Borders	1990	1990	Female	Mandible
NS.1994.007.001	Culbin Spruce Plantation	North Scotland	1967	1960	Male	Orbit, Zygomatic Arch
NS.1994.077.001	Culbin Spruce Plantation	North Scotland	1967	1960	Male	Temporalis Scar, Mandible
NS.1994.077.004	Newton Roseisle Forest	North Scotland	1967	1960	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.1994.077.005	Culbin Spruce Plantation	North Scotland	1967	1960	Male	Mandible
NS.1994.077.006	Culbin Spruce Plantation	North Scotland	1967	1960	Male	Mandible
NS.1994.078	Manor Valley, Peebles	Borders	1985	1980	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.1994.079	Dunkeld North Scotland?	North Scotland	1992	1990	Male	Mandible
NS.1994.08	Blaich near Ft. William	North Scotland	1989	1980	Female	Mandible
NS.1994.096	Hayton Carlisle	Borders	1994	1990	Male	Mandible
NS.1995.061	Nr Clatto Hill Fife	North Scotland	1990	1990	Male	Mandible
NS.1995.062.001	Elie Fife	North Scotland	1994	1990	Male	Dorsal Midline, Orbit, Zygomatic Arch, Mandible
NS.1995.062.002		North Scotland	1994	1990	Female	Dorsal Midline, Orbit, Temporalis Scar
NS.1995.062.003	Elie Fife	North Scotland	1994	1990	Male	Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.1995.062.004	Elie Fife	North Scotland	1994	1990	Female	Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.1995.062.005	Elie Fife	North Scotland	1994	1990	Female	Mandible
NS.1995.063.002	Aviemore	North Scotland	1994	1990	Female	Dorsal Midline, Orbit, Zygomatic Arch, Mandible
NS.1995.063.003	NH 939 189	North Scotland	1994	1990	Female	Mandible
NS.1995.063.004	Boat of Garten	North Scotland	1994	1990	Male	Mandible
NS.1995.063.005	Granton on Spey	North Scotland	1994	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.1995.063.006	between Polchar and Lochan Eilein	North Scotland	1994	1990	Male	Mandible

NS.1995.063.011	Kinhurdy Road Achintowl	North Scotland	1994	1990	Male	Mandible
NS.1995.064	Tomatin	North Scotland	1992	1990	Female	Mandible
NS.1995.065	Braemar	North Scotland	1988	1980	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.063.009	Badenoch and Strathinspay	North Scotland	1994	1990	Male	Mandible
NS.2000.063.010	Boat of Garten	North Scotland	1994	1990	Female	Mandible
NS.2000.189.003	Jersey	Jersey	1998	1990	Female	Mandible
NS.2000.189.004	Jersey	Jersey	1998	1990	Female	Mandible
NS.2000.189.005	Jersey	Jersey	1998	1990	Female	Mandible
NS.2000.189.006	Jersey	Jersey	1997	1990	Male	Mandible
NS.2000.189.008	Jersey	Jersey	1997	1990	Female	Mandible
NS.2000.189.013	Jersey	Jersey	1996	1990	Female	Mandible
NS.2000.189.014	Jersey	Jersey	1997	1990	Male	Mandible
NS.2000.189.015	Jersey	Jersey	1993	1990	Male	Mandible
NS.2000.189.016	Jersey	Jersey	1997	1990	Male	Mandible
NS.2000.189.017	Jersey	Jersey	1995	1990	Female	Mandible
NS.2000.189.018	Jersey	Jersey	1997	1990	Female	Mandible
NS.2000.189.019	Jersey	Jersey	1996	1990	Female	Mandible
NS.2000.189.020	Jersey	Jersey	1997	1990	Female	Mandible
NS.2000.189.022	Jersey	Jersey	1996	1990	Male	Mandible
NS.2000.189.024	Jersey	Jersey	1997	1990	Female	Mandible
NS.2000.189.026	Jersey	Jersey	1998	1990	Male	Mandible
NS.2000.189.028	Jersey	Jersey	1998	1990	Female	Mandible
NS.2000.189.029	Jersey	Jersey	1998	1990	Male	Mandible
NS.2000.189.030	Jersey	Jersey	1998	1990	Male	Mandible
NS.2000.194.003	formby, Lancs	Formby	1995	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.006	SD 368 148 Lancashire	Formby	1994	1990	Female	Dorsal Midline, Orbit, Zygomatic Arch, Mandible
NS.2000.194.008	Formby Lancs	Formby	1995	1990	Male	Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.009	Formby Lancs	Formby	1995	1990	Male	Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.010	Becraigs Linlithgow	Borders	1995	1990	Male	Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.011	Becraigs Linlithgow	Borders	1995	1990	Female	Mandible
NS.2000.194.012	Westmoreland	Borders	1995	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch
NS.2000.194.013	Formby Lancs	Formby	1994	1990	Female	Mandible
NS.2000.194.014	Keswick Cumbria	Borders	1994	1990	Male	Dorsal Midline, Zygomatic Arch, Mandible

NS.2000.194.015	Summers How SD392878	Borders	1994	1990	Male	Mandible
NS.2000.194.016	Keswick, Cumbria	Borders	1994	1990	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.017	Formby	Formby	1994	1990	Male	Mandible
NS.2000.194.018	Stannington	Borders	1995	1990	Male	Temporalis Scar, Mandible
NS.2000.194.019	Windermere and Newby Bridge	Borders	1996	1990	Male	Mandible
NS.2000.194.020	Cumbria	Borders	1995	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch
NS.2000.194.022		North Scotland	1995	1990	Female	Dorsal Midline, Temporalis Scar
NS.2000.194.023		North Scotland	Unknown	Unknown	Female	Orbit, Temporalis Scar, Zygomatic Arch
NS.2000.194.024	Ulverston, Cumbria	Borders	1994	1990	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.025	Keswick Cumbria	Borders	1995	1990	Female	Mandible
NS.2000.194.026	Cumbria	Borders	1994	1990	Female	Mandible
NS.2000.194.030	Cumbria SD396938	Borders	1995	1990	Male	Mandible
NS.2000.194.031	Coniston, Cumbria	Borders	1995	1990	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.033	Beecraigs, Linlithgow	Borders	1995	1990	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.034	Cumbria	Borders	1995	1990	Male	Mandible
NS.2000.194.037	Formby Lancs	Formby	1995	1990	Female	Mandible
NS.2000.194.038	Nr. Nairn	North Scotland	1995	1990	Male	Mandible
NS.2000.194.039	Cumbria	Borders	1995	1990	Female	Mandible
NS.2000.194.044	Cumbria	Borders	1995	1990	Female	Mandible
NS.2000.194.045	Formby Lancs	Formby	1995	1990	Female	Dorsal Midline, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.046	Linlithgow from Elie	North Scotland	1995	1990	Male	Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.049	Nr Keswick Cumbria	Borders	1994	1990	Unknown	Mandible
NS.2000.194.055	Borrowdale Cumbria	Borders	1994	1990	Male	Mandible
NS.2000.194.056	Cumbria	Borders	1995	1990	Male	Mandible
NS.2000.194.061	Lancashire	Formby	1995	1990	Male	Dorsal Midline, Orbit, Temporalis Scar, Mandible
NS.2000.194.063	ormskirk, lancs	Formby	1995	1990	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.064	Ruff Woods, Ormskirk	Formby	1995	1990	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar,

						Zygomatic Arch, Mandible
NS.2000.194.065	Manesty Caravan Site, Keswick	Borders	1995	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.066	South of Bowness on windermere	Borders	1996	1990	Male	Mandible
NS.2000.194.067	Nr Selkirk Galashiels	Borders	1996	1990	Male	Mandible
NS.2000.194.068	Derwent Gorge	Borders	1996	1990	Female	Dorsal Midline, Temporalis Scar, Mandible
NS.2000.194.069	Formby Lancs	Formby	1995	1990	Female	Mandible
NS.2000.194.073	Stair, Keswick, Cumbria	Borders	1996	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.078	Holyware Coniston Cumbria	Borders	1996	1990	Male	Dorsal Midline, Orbit, Zygomatic Arch, Mandible
NS.2000.194.079	Rd from Watermillock to Pooley Bridge Cumbria	Borders	1996	1990	Female	Mandible
NS.2000.194.081	108 Sd 275080 Formby	Formby	1994	1990	Female	Mandible
NS.2000.194.082	Swittlegarth Ireby Carlisle	Borders	1994	1990	Male	Mandible
NS.2000.194.083	Brigsteer Cumbria	Borders	1994	1990	Male	Dorsal Midline, Orbit, Zygomatic Arch, Mandible
NS.2000.194.084	Formby point caravan site	Formby	1994	1990	Male	Mandible
NS.2000.194.086	Lazonby, Penrith, Cumbria	Borders	1996	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.087	A6 South of Hackthorpe	Borders	1996	1990	Female	Mandible
NS.2000.194.093	Roudsea Woods, South Cumbria	Borders	1996	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch
NS.2000.194.095	Nr. Lamb How Windermere	Borders	1996	1990	Female	Mandible
NS.2000.194.098	Windermere Rd Cumbria	Borders	1995	1990	Male	Dorsal Midline, Temporalis Scar, Mandible
NS.2000.194.099	Ruff Woods, Ormskirk	Formby	1995	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.10	Beecraigs Linlithgow	Borders	1995	1990	Male	Mandible
NS.2000.194.100	Ruff Woods Ormskirk Lancs	Formby	1995	1990	Male	Mandible
NS.2000.194.101	Nr River Brathay West of Ambleside Cumbria	Borders	1995	1990	Male	Mandible
NS.2000.194.102	Formby Lancs	Formby	1995	1990	Male	Mandible
NS.2000.194.103	Lockerbie, Dumfriesshire	Borders	1995	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.104	south of Beech hill hotel Cumbria	Borders	1995	1990	Female	Mandible

NS.2000.194.105	Whomcatterpass Nr Keswick Cumbria	Borders	1996	1990	Male	Mandible
NS.2000.194.108	Stratheden Nr Cupar	North Scotland	1995	1990	Male	Mandible
NS.2000.194.109	Ruff Woods Ormskirk Lancs	Formby	1996	1990	Male	Dorsal Midline, Orbit, Temporalis Scar, Mandible
NS.2000.194.110	Ruff Wood Ormskirk Lancs	Formby	1995	1990	Male	Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.111	lancashire	Formby	1995	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch
NS.2000.194.112	mere Sands Wood Lancashire GR SD 446155	Formby	1995	1990	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.113	Cann Hill graythraite Newby Bridge Nr Ulverston	Borders	1995	1990	Male	Mandible
NS.2000.194.116	NZ 16NW GR NZ125659	Borders	1996	1990	Female	Mandible
NS.2000.194.117						Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.118	Cumbria	Borders	1995	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.119	Broomholme, Langholm	Borders	1995	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.124	Whinlatter Forest Cumbria	Borders	1995	1990	Female	Temporalis Scar, Mandible
NS.2000.194.125	mere sands wood, rufford, lancashire	Formby	1995	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.134	Derwent Gorge	Borders	1996	1990	Male	Dorsal Midline, Orbit, Temporalis Scar, Mandible
NS.2000.194.138	Blackhorse Wood, Nr Wolsingham, Co Durham	Borders	1996	1990	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.139	Ravenmeals Lane Formby Lancs	Formby	1995	1990	Male	Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.140	Ravenmeols Lane, Formby, Lancs	Formby	1996	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.194.33	Beebraigs Linlithgow	Borders	1995	1990	Male	Mandible
NS.2000.194.45	Formby	Formby	1995	1990	Female	Mandible
NS.2000.194.61	Lancashire	Formby	1995	1990	Unknown	Mandible
NS.2000.194.64	Ruff Woods Ormskirk Lancs	Formby	1995	1990	Male	Mandible
NS.2000.194.8	Formby Lancs	Formby	1995	1990	Male	Mandible
NS.2000.194.9	Formby Lancs	Formby	1995	1990	Male	Mandible
NS.2000.194.99	Ruff Woods Ormskirk Lancs	Formby	1995	1990	Female	Mandible

NS.2000.195.001	Formby	Formby	1993	1990	Male	Dorsal Midline, Orbit, Temporalis Scar, Mandible
NS.2000.195.002	Formby	Formby	1993	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.195.003		Borders	1994	1990	Unknown	Dorsal Midline, Temporalis Scar, Zygomatic Arch
NS.2000.195.004	Formby	Formby	1992	1990	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.195.005	Witherslack Cumbria	Borders	1994	1990	Female	Dorsal Midline, Temporalis Scar, Mandible
NS.2000.195.006	Formby	Formby	1993	1990	Female	Dorsal Midline, Orbit, Temporalis Scar, Mandible
NS.2000.195.007	Langholm Ae	Borders	1994	1990	Male	Mandible
NS.2000.195.010	Formby	Formby	1993	1990	Male	Mandible
NS.2000.195.012	Formby	Formby	1993	1990	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.195.013	Witherslack, Cumbria	Borders	1994	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.195.014	Windemere Cumbria	Borders	1993	1990	Female	Mandible
NS.2000.195.015	Blairgowrie	North Scotland	1993	1990	Male	Mandible
NS.2000.195.017	Abbey St Bathans Berwickshire	Borders	1994	1990	Female	Mandible
NS.2000.195.018	Peil Wyke Cumbria	Borders	1993	1990	Male	Mandible
NS.2000.195.019	Formby	Formby	1993	1990	Female	Mandible
NS.2000.195.020	Formby	Formby	1995	1990	Male	Mandible
NS.2000.195.021	Rothbury	Borders	1991	1990	Male	Mandible
NS.2000.195.022	Peebles	Borders	1995	1990	Unknown	Mandible
NS.2000.195.024	Eglingham Northumberland	Borders	1992	1990	Female	Mandible
NS.2000.195.025	Peebles	Borders	1997	1990	Female	Mandible
NS.2000.195.026	Cumbria	Borders	1993	1990	Female	Dorsal Midline, Orbit, Zygomatic Arch, Mandible
NS.2000.195.027	Peil Wyke, Cumbria	Borders	1991	1990	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.195.028	Arnside Knott	Borders	1989	1980	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.195.029	Formby	Formby	1994	1990	Male	Mandible
NS.2000.195.030	Formby	Formby	1993	1990	Female	Mandible
NS.2000.195.032	Formby	Formby	1993	1990	Female	Mandible
NS.2000.195.034	Formby	Formby	1993	1990	Female	Mandible
NS.2000.195.035	Formby	Formby	1994	1990	Female	Mandible

NS.2000.195.036	Formby	Formby	1991	1990	Male	Mandible
NS.2000.195.037	Formby	Formby	1992	1990	Male	Mandible
NS.2000.195.038	Formby	Formby	1993	1990	Female	Mandible
NS.2000.195.039	Formby	Formby	1993	1990	Male	Mandible
NS.2000.195.041	Formby	Formby	1993	1990	Male	Mandible
NS.2000.195.043	Linholme Cumbria	Borders	1993	1990	Male	Mandible
NS.2000.195.044	Elie	North Scotland	1991	1990	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.195.045	Formby	Formby	1995	1990	Male	Mandible
NS.2000.195.046	Formby	Formby	1993	1990	Male	Mandible
NS.2000.195.050	Penrith, Cumbria	Borders	1991	1990	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.195.051	Formby	Formby	1991	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.195.053	Silverdale Lancashire	Formby	1991	1990	Female	Mandible
NS.2000.195.054	Whinlatter Cumbria	Borders	1991	1990	Unknown	Mandible
NS.2000.195.055	Whinlatter CUMBria	Borders	1991	1990	Unknown	Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.195.056	Formby	Formby	1995	1990	Female	Mandible
NS.2000.195.057	Elie	North Scotland	1991	1990	Male	Mandible
NS.2000.195.060	Arnside	Borders	1990	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.195.063	Cowal	North Scotland	1993	1990	Female	Mandible
NS.2000.195.066	Formby	Formby	1994	1990	Female	Mandible
NS.2000.195.069	Ferness Nr. Nairn	North Scotland	1988	1980	Male	Dorsal Midline, Temporalis Scar, Mandible
NS.2000.195.071	Inverness	North Scotland	1989	1980	Male	Mandible
NS.2000.195.072	Easter Ross F district Blackisle	North Scotland	1990	1990	Female	Mandible
NS.2000.195.073	Hesleside Kielder	Borders	1993	1990	Female	Mandible
NS.2000.195.075	Isle of Arran	North Scotland	1997	1990	Male	Mandible
NS.2000.195.077.	Elie	North Scotland	1990	1990	Female	Mandible
NS.2000.195.078	Elie	North Scotland	1990	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.195.22		Borders	1995	1990	Female	Dorsal Midline, Orbit, Zygomatic Arch
NS.2000.195.51	Formby	Formby	1991	1990	Female	Mandible
NS.2000.238.001	Loch of the Lowes	North Scotland	1981	1980	Female	Mandible
NS.2000.238.004	Duncow Estate	Borders	1988	1980	Female	Mandible
NS.2000.238.007	Culbin	North Scotland	1967	1960	Male	Mandible

NS.2000.238.008	Newton Roseisle Forest	North Scotland	1967	1960	Male	Mandible
NS.2000.238.009	Culbin	North Scotland	1967	1960	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch
NS.2000.238.010	Newton Roseisle Forest	North Scotland	1967	1960	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.238.011	Newton Roseisle Forest	North Scotland	1969	1960	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.238.012	Culbin	North Scotland	1967	1960	Female	Mandible
NS.2000.238.013	culbin	North Scotland	1967	1960	Female	Mandible
NS.2000.238.017	Sorn Muirkirk Road Ayrshire	Borders	1991	1990	Male	Mandible
NS.2000.238.018	Tentsmuir Fife	North Scotland	1970	1970	Female	Mandible
NS.2000.258	Cumbria NY511189	Borders	2000	2000	Male	Mandible
NS.2000.259	Balgrove	North Scotland	1995	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.261.002	Nu003497	Borders	1992	1990	Unknown	Mandible
NS.2000.263.001	Abernally	North Scotland	1995	1990	Female	Mandible
NS.2000.263.002	Moormove Road	North Scotland	1995	1990	Male	Mandible
NS.2000.263.007	Mabie Forest	Borders	1996	1990	Male	Mandible
NS.2000.263.010	Blackmill Woods	North Scotland	1997	1990	Female	Mandible
NS.2000.263.011	West Terrace, Kingussie	North Scotland	1998	1990	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.263.012	nr Perth	North Scotland	1997	1990	Male	Mandible
NS.2000.264.003	Mullingarroch	North Scotland	1997	1990	Male	Mandible
NS.2000.275	Waterside Ringford Galloway	Borders	1997	1990	Male	Mandible
NS.2000.277	Blair Castle Blair Atholl Perthshire	North Scotland	1996	1990	Female	Mandible
NS.2000.283.001	formby Town	Formby	1997	1990	Male	Mandible
NS.2000.283.002	Formby	Formby	1997	1990	Male	Mandible
NS.2000.283.003	Formby	Formby	1996	1990	Female	Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.283.003a	Blamorie Carpark Beecraigs Country Park West Lothian	Borders	1994	1990	Female	Mandible
NS.2000.283.004	Duncow Estate	Borders	1996	1990	Female	Mandible
NS.2000.283.006	Formby	Formby	1996	1990	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.283.007	Culbin	North Scotland	1996	1990	Female	Mandible
NS.2000.284	Garden of Kirrongtree lodge	Borders	1999	1990	Female	Mandible

	Nw Galloway Rd Newton Stewart					
NS.2000.285.001	Craig Dunaun Hospital, Inverness	North Scotland	1995	1990	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.285.002	Nethybridge	North Scotland	1994	1990	Female	Mandible
NS.2000.286	Highland Wildlife Park	North Scotland	1996	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.29	eglingham Nr Alnwick Northumberland	Borders	1990	1990	Male	Mandible
NS.2000.291.001	Abernethy Forest between Forest Lodge and Nethybridge	North Scotland	1994	1990	Female	Mandible
NS.2000.291.002	Abernethy Forest, b/w Forest Lodge & Nethybridge	North Scotland	1991	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.292.001	Nr Kirkpatrick, Durham	Borders	1994	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2000.292.002	Abernathy Forest (between forest lodge and nethybridge)	North Scotland	1994	1990	Male	Mandible
NS.2000.293	Croy Inverness	North Scotland	1997	1990	Male	Dorsal Midline, Temporalis Scar, Mandible
NS.2000.294	Nr Kirkpatrick Durham	Borders	1997	1990	Male	Mandible
NS.2000.407	Gosforth Cumbria	Borders	2000	2000	Male	Mandible
NS.2000.431	CrossRoads between Morland and Gt Strickland, near Penrith, Cumbria	Borders	2000	2000	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2001.152	NY 452225	Borders	2001	2000	Male	Mandible
NS.2001.153	Crosslands Graythwaite Rd S.Cumbria	Borders	2000	2000	Male	Mandible
NS.2003.107.001	Jersey	Jersey	2000	2000	Female	Mandible
NS.2003.107.003	Jersey	Jersey	2000	2000	Female	Mandible
NS.2003.107.005	Jersey	Jersey	1999	1990	Male	Mandible
NS.2003.107.006	Jersey	Jersey	1998	1990	Female	Mandible
NS.2003.107.007	Jersey	Jersey	1999	1990	Female	Mandible
NS.2003.107.008	Jersey	Jersey	1999	1990	Female	Mandible
NS.2003.107.009	Jersey	Jersey	1999	1990	Female	Mandible
NS.2003.107.010	Jersey	Jersey	1998	1990	Male	Mandible
NS.2003.107.012	Jersey	Jersey	2001	2000	Male	Mandible
NS.2003.107.013	Jersey	Jersey	2000	2000	Male	Mandible
NS.2003.107.014	Jersey	Jersey	1999	1990	Female	Mandible
NS.2003.107.016	Jersey	Jersey	1999	1990	Male	Mandible
NS.2004.041	B9131 out Stravithie Dunino Fife	North Scotland	2002	2000	Male	Mandible
NS.2004.211.001		North Scotland	2004	2000	Female	Dorsal Midline, Orbit

NS.2004.211.002	Drumshallech Croft Banchory Kincardineshire	North Scotland	2004	2000	Female	Dorsal Midline, Orbit, Mandible
NS.2004.211.1	Drumshalloch Croft Banchory Kincardineshire	North Scotland	2004	2000	Male	Mandible
NS.2004.244	Scotland, Aberdeenshire, Deebank, Gordondale, Banchory	North Scotland	2001	2000	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.2006.003		North Scotland	2005	2000	Male	Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch
NS.2006.004	Moncreiffe Hill Perthshire	North Scotland	2001	2000	Unknown	Mandible
NS.2006.3	Scotland Aberdeenshire Deebank Gordondale Banchory	North Scotland	2001	2000	Male	Mandible
NS.95.06	Boat of Garten	North Scotland	1994	1990	Female	Mandible
NS.95.062.001		North Scotland	1994	1990	Unknown	Temporalis Scar
NS.95.09	Abernethy	North Scotland	1994	1990	Female	Mandible
NS.95.1	Nethybridge	North Scotland	1994	1990	Male	Mandible
NS.95.11	Nethybridge	North Scotland	1994	1990	Female	Mandible
NS.95.16	Morland and Gt.Strick land Penrith Cumbria	Borders	1994	1990	Female	Mandible
NS.GH114.09	Nawtonmore Rd / Laggan Rd	North Scotland	2008	2000	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.GH115.09	White Dene Reservoir Northumberland	Borders	2005	2000	Male	Mandible
NS.GH117.09	SNH Nit 989974, Rattuen Rd Raltia	North Scotland	2007	2000	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.GH118.09	Edinburgh	Borders	2003	2000	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.GH152.09	Granton Rd kill	Borders	2007	2000	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.GH153.09	NO671969	North Scotland	2007	2000	Male	Mandible
NS.GH155.09	Rothiemurchus	North Scotland	2000	2000	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.GH156.09	Entrance of Forrest Lodge	North Scotland	2001	2000	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.GH158.09	NH967193	North Scotland	2001	2000	Female	Mandible
NS.GH160.09	Darva Road 1 mile N of Grantown on	North Scotland	1994	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar,

	Spey by railway bridge					Zygomatic Arch, Mandible
NS.GH166.09	Pitmuies House	North Scotland	1989	1980	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.GH167.09	hamirton	Borders	1999	1990	Male	Mandible
NS.GH168.09	Sandoe Hall Hexham	Borders	2001	2000	Male	Mandible
NS.GH171.09	Falstone	Borders	2000	2000	Male	Mandible
NS.GH172.09	Fearnant Lawers Village North Loch Tay	North Scotland	1995	1990	Male	Mandible
NS.GH174.09	A762 at Dochrae Bank House Hensol Castle Douglas	Borders	2001	2000	Female	Mandible
NS.GH181.09		Borders	1994	1990	Male	Dorsal Midline, Orbit, Temporalis Scar
NS.GH182.09	Banchory Kincardineshire AB31 4DD	North Scotland	2006	2000	Male	Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.GH185.09	Glen Isla Brewlands Bridge Clacknochater	North Scotland	2003	2000	Female	Dorsal Midline, Temporalis Scar, Zygomatic Arch, Mandible
NS.GH187.09	Glen Tanar	North Scotland	1984	1980	Female	Mandible
NS.GH188.09	Loch Davan, Muir of Dinnet	North Scotland	1996	1990	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.GH189.09	Glassel	North Scotland	1994	1990	Male	Mandible
NS.GH196.09	Loch Davan	North Scotland	1988	1980	Male	Mandible
NS.GH197.09	Embleton moor Northumberland	Borders	2000	2000	Female	Mandible
NS.GH198.09	Cawdor Nairn	North Scotland	2002	2000	Female	Mandible
NS.GH199.09	Jersey	Jersey	2001	2000	Male	Mandible
NS.GH218.09	Jersey	Jersey	2001	2000	Male	Mandible
NS.GH219.09	Jersey	Jersey	2001	2000	Female	Mandible
NS.GH222.09	Jersey	Jersey	2001	2000	Female	Mandible
NS.GH224.09	Jersey	Jersey	2001	2000	Female	Mandible
NS.GH226.09	Jersey	Jersey	2001	2000	Female	Mandible
NS.GH240.14	Grid Ref NO325068	North Scotland	2011	2010	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch
NS.GP16	Newton Roseisle Forest	North Scotland	1967	1960	Male	Mandible
NS.M2003.92	Clephanton m Highlands	North Scotland	1992	1990	Male	Mandible
NS.R115.05	White Dene Resrvoir, Northumberland	Borders	1995	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch
NS.R177.98	Nr. Nairn (A939)	North Scotland	1996	1990	Female	Mandible
NS.R223.98	St Andrews, Fife	North Scotland	1996	1990	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.R249.98	Glenbranter	North Scotland	1998	1990	Female	Whole Cranium, Dorsal Midline, Orbit,

						Temporalis Scar, Zygomatic Arch
NS.R4499	Argyll Arran	North Scotland	1997	1990	Male	Mandible
NS.R5199	Arran Argyll	North Scotland	1997	1990	Male	Mandible
NS.R5299	Arran Argyll	North Scotland	1997	1990	Male	Mandible
NS.R5399	Arran Argyll Cnockan Gorge	North Scotland	1996	1990	Female	Mandible
NS.RL38.96		North Scotland	1995	1990	Male	Dorsal Midline, Temporalis Scar
NS.SR10.20	Formby proper	Formby	2011	2010	Male	Mandible
NS.SR100.20	Formby proper	Formby	2012	2010	Male	Mandible
NS.SR101.20	Formby	Formby	2014	2010	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR102.20	Formby	Formby	2014	2010	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch
NS.SR102.30	Formby proper	Formby	2014	2010	Male	Mandible
NS.SR103.20	Formby proper	Formby	2014	2010	Female	Mandible
NS.SR104		Formby	2013	2010	Female	Zygomatic Arch
NS.SR104.20	Formby	Formby	2013	2010	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Mandible
NS.SR105.20	Formby	Formby	2014	2010	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR106.20	Formby proper	Formby	2014	2010	Female	Dorsal Midline, Orbit, Zygomatic Arch, Mandible
NS.SR107.20	Formby	Formby	2013	2010	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR109.20	Formby proper	Formby	2014	2010	Male	Mandible
NS.SR110.20	Formby	Formby	2016	2010	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR111.20	Formby proper	Formby	2014	2010	Male	Mandible
NS.SR112.20	Formby	Formby	2014	2010	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR114.20	Formby proper	Formby	2013	2010	Male	Mandible
NS.SR117.20	Formby proper	Formby	2013	2010	Female	Mandible
NS.SR118.20	Formby	Formby	2012	2010	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR119.20	Formby proper	Formby	2014	2010	Male	Dorsal Midline, Temporalis Scar, Mandible
NS.SR119.50		Formby	2014	2010	Male	Orbit, Zygomatic Arch
NS.SR120.20	Formby proper	Formby	2012	2010	Female	Mandible

NS.SR121		Formby	2011	2010	Female	Dorsal Midline
NS.SR121.20	Formby	Formby	2011	2010	Female	Whole Cranium, Orbit, Temporalis Scar, Zygomatic Arch
NS.SR122.20	Formby	Formby	2011	2010	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR125.20	Formby proper	Formby	2011	2010	Male	Mandible
NS.SR127.20	Formby	Formby	2012	2010	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR128.20	Formby proper	Formby	2012	2010	Male	Dorsal Midline, Orbit, Zygomatic Arch, Mandible
NS.SR129.20	Formby proper	Formby	2011	2010	Male	Mandible
NS.SR13.20		Formby	2011	2010	Unknown	Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch
NS.SR131.20	Formby	Formby	2011	2010	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR132.20	Formby	Formby	2012	2010	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR134.20	Formby proper	Formby	2011	2010	Male	Mandible
NS.SR135.20	Formby	Formby	2016	2010	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch
NS.SR136.20	Formby proper	Formby	2013	2010	Male	Mandible
NS.SR137.20	Formby proper	Formby	2011	2010	Male	Dorsal Midline, Mandible
NS.SR14.20	Formby proper	Formby	2012	2010	Female	Mandible
NS.SR140.20	Formby proper	Formby	2011	2010	Male	Mandible
NS.SR144.20	Formby proper	Formby	2012	2010	Female	Dorsal Midline, Mandible
NS.SR146.20	Formby	Formby	2012	2010	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR148.20	Formby proper	Formby	2014	2010	Female	Dorsal Midline, Orbit, Mandible
NS.SR151.20	Formby	Formby	2014	2010	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR155.20	Formby	Formby	2014	2010	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR156.20	Formby	Formby	2014	2010	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch
NS.SR159.20	Formby proper	Formby	2014	2010	Female	Mandible
NS.SR160.20	Formby	Formby	2012	2010	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible

NS.SR161.20	Formby	Formby	2014	2010	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR162.20	Formby proper	Formby	2012	2010	Female	Dorsal Midline, Orbit, Temporalis Scar, Mandible
NS.SR163.20	Formby proper	Formby	2011	2010	Female	Mandible
NS.SR164.20	Formby proper	Formby	2018	2010	Female	Mandible
NS.SR165.20	Formby	Formby	2013	2010	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR166.20	Formby proper	Formby	2014	2010	Male	Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR166.50	Formby	Formby	2014	2010	Male	Whole Cranium, Zygomatic Arch
NS.SR167.20	Formby proper	Formby	2013	2010	Male	Mandible
NS.SR168.20	Formby proper	Formby	2012	2010	Female	Temporalis Scar, Mandible
NS.SR169.20	Formby	Formby	2011	2010	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR17.20	Formby	Formby	2011	2010	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR170.20	Formby	Formby	2015	2010	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR172.20	Formby	Formby	2017	2010	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR173.20	Formby proper	Formby	2016	2010	Female	Mandible
NS.SR175.20	Formby proper	Formby	2016	2010	Female	Mandible
NS.SR176.20	Formby proper	Formby	2016	2010	Male	Mandible
NS.SR179.20	Formby	Formby	2018	2010	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR18		Formby	2011	2010	Female	Dorsal Midline
NS.SR18.20	Formby	Formby	2011	2010	Female	Whole Cranium, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR180.20	Formby	Formby	2018	2010	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR181.20	Formby	Formby	2018	2010	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR183.20	Formby proper	Formby	2014	2010	Male	Mandible
NS.SR19.20	Formby	Formby	2012	2010	Male	Whole Cranium, Dorsal Midline, Orbit,

						Temporalis Scar, Zygomatic Arch, Mandible
NS.SR20.20	Formby proper	Formby	2013	2010	Male	Mandible
NS.SR22.20	Formby proper	Formby	2012	2010	Male	Mandible
NS.SR23.20	Formby proper	Formby	2012	2010	Male	Mandible
NS.SR24.20	Formby	Formby	2011	2010	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR25.20	Formby	Formby	2016	2010	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR27.20	Formby proper	Formby	2012	2010	Male	Mandible
NS.SR29.20	Formby proper	Formby	2011	2010	Male	Mandible
NS.SR30.20	Formby	Formby	2011	2010	Male	Whole Cranium, Temporalis Scar, Mandible
NS.SR32.20		Formby	2011	2010	Male	Dorsal Midline, Orbit, Zygomatic Arch
NS.SR35.20	Formby proper	Formby	2014	2010	Female	Mandible
NS.SR36.20	Formby proper	Formby	2014	2010	Male	Mandible
NS.SR37.20	Formby	Formby	2014	2010	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR39.20	Formby proper	Formby	2014	2010	Female	Mandible
NS.SR41.20	Formby	Formby	2014	2010	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR42.20	Formby	Formby	2014	2010	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR44.20	Formby	Formby	2014	2010	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR45.20	Formby proper	Formby	2014	2010	Female	Temporalis Scar, Mandible
NS.SR46.20	Formby proper	Formby	2014	2010	Female	Mandible
NS.SR47.20	Formby proper	Formby	2014	2010	Male	Mandible
NS.SR49.20	Formby proper	Formby	2014	2010	Female	Mandible
NS.SR50.20	Formby proper	Formby	2014	2010	Female	Mandible
NS.SR51.20	Formby proper	Formby	2014	2010	Male	Mandible
NS.SR52.20	Formby proper	Formby	2014	2010	Male	Mandible
NS.SR54.20	Formby	Formby	2014	2010	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR57.20	Formby proper	Formby	2013	2010	Female	Mandible
NS.SR58.20	Formby proper	Formby	2018	2010	Female	Mandible
NS.SR59.20		Formby	2014	2010	Male	Dorsal Midline, Orbit, Temporalis Scar
NS.SR62.20	Formby	Formby	2014	2010	Female	Whole Cranium, Dorsal Midline, Orbit,

						Temporalis Scar, Zygomatic Arch, Mandible
NS.SR63.20	Formby proper	Formby	2013	2010	Male	Mandible
NS.SR64.20	Formby proper	Formby	2013	2010	Male	Mandible
NS.SR65.20	Formby	Formby	2013	2010	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR66.20	Formby proper	Formby	2014	2010	Male	Mandible
NS.SR67.20	Formby proper	Formby	2013	2010	Male	Mandible
NS.SR68.20	Formby proper	Formby	2013	2010	Female	Mandible
NS.SR69.20	Formby proper	Formby	2014	2010	Male	Mandible
NS.SR70.20	Formby proper	Formby	2013	2010	Male	Mandible
NS.SR73.20	Formby	Formby	2011	2010	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR75.20	Formby proper	Formby	2013	2010	Male	Mandible
NS.SR76.20	Formby	Formby	2014	2010	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR77.20	Formby	Formby	2013	2010	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR78.20	Formby	Formby	2013	2010	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR79.20	Formby proper	Formby	2013	2010	Female	Mandible
NS.SR80.20	Formby proper	Formby	2013	2010	Male	Mandible
NS.SR81.20	Formby	Formby	2015	2010	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR82.20	Formby proper	Formby	2014	2010	Male	Mandible
NS.SR85.20	Formby	Formby	2014	2010	Female	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch, Mandible
NS.SR86.20	Formby	Formby	2013	2010	Male	Whole Cranium, Dorsal Midline, Orbit, Temporalis Scar, Zygomatic Arch
NS.SR90.20	Formby proper	Formby	2013	2010	Male	Mandible
NS.SR98.20	Formby proper	Formby	2013	2010	Female	Mandible
NS.SR99.20	Formby proper	Formby	2014	2010	Male	Mandible

Appendix 2. Raw Data of Analysis of cut and whole nut samples from Chapter 3.

Included as separate Excel document Appendix 2.xls

Appendix 3. Nut Collecting Record Sheet, designed for use by Schools involved in the Citizen Science Project funded by the Royal Society APEX public engagement grant awarded to Dr Philip Cox. Attached as a separate PDF document Appendix 3.pdf.