Evaluating the dietary micro-remain record in dental calculus and its application in deciphering hominin diets in Palaeolithic Eurasia

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Introduction: the evolutionary context of Neanderthal dietary ecology

2.1 The importance of understanding Palaeolithic diets

Energy provisioning is central to mammalian ecology. Understanding mammalian life requires examining its acquisition of energy. Dietary details, such as nutritional requirements, use of different environments, and trophic level may define a taxon (Hutchinson, 1959; Armstrong and McGehee, 1980). The importance of diet is no less true for our own lineage (Winterhalder and Smith, 2000). Dietary strategies are relevant for understanding the most basic and the most specialised level of organisation of human societies. Exposure to new environments and climatic cycles forced major subsistence changes because these presented energetic challenges that restricted, expanded, or otherwise shaped past populations. Scholars recognise that reconstructing ancient dietary niches is of central importance to early hominin palaeobiology. Many authors have suggested that niche switching is linked to landmark changes in hominin evolutionary history (O'Connell et al., 1999; Wrangham, 2000). This is evident from the emergence of distinct morphological and behavioural changes.

Among early Pleistocene hominins, for example, differences in the apparatus of mastication distinguish robust and gracile forms. Enormous, thickly enamelled teeth and robust mandibles that supported powerful mastication ability separate robust *Paranthropus boisei* and *Paranthropus robustus* from the less-developed forms including *Australopithecus afarensis* and *Australopithecus africanus*. Chipping of the enamel from the robust species is suggestive of the consumption of hard foods (Constantino et al., 2010), although microwear fails to support this view (Ungar et al., 2008). The ability to consume extremely hard and tough foods may have been an adaptation for processing fallback foods during seasonal bouts of food scarcity (Lee-Thorp et al., 1994; Ungar et al., 2008; Constantino et al., 2010). Stable isotopic evidence has told a different story on why this specialised robust anatomy evolved. *Paranthropus robustus* isotopic values suggest this hominin was reliant on C_4 grasses and sedges, in contrast to those of nearly all other hominins, which show more reliance on C_3 resources. This implies that the robust craniodental features are an adaption to "repetitive loading" from consuming large quantities of low-quality vegetation rather than hard objects (Cerling et al., 2011). These craniodental traits represent differences in adaptive capabilities from more gracile forms even if their dietary niches were composed of similarly tough foods (Laden and Wrangham, 2005; Ungar et al., 2008).

Just as early Pleistocene robust hominins morphologically adapted to a dietary niche, other hominins have evolved morphologically in many other ways because of exploiting new dietary regimes. A dietary change is intertwined with the most dramatic externalisation of hominin evolution, the dramatic increased size of the hominin brain. According to the "expensive tissue hypothesis," the emergence of a large and energetically costly large brain and small gut in the *Homo* genus arose concomitant to an increasingly energy-dense diet (Aiello and Wheeler, 1995) . The trade-off between a larger brain size and a smaller digestive tract demanded a higher quality diet, which was possible through either consuming more high-energy classes of food or through external digestion (i.e. food processing or cooking). Some of the most recent anatomical changes in hominin history relate to diet. Present day human dentition is the outcome of a progressive reduction in the size and number of teeth since the first emergence of *Homo*. The process of diminishing tooth size accelerated in periods of dietary change such as the transition to agriculture (Loring Brace et al., 1987). A decrease in the loads exerted on teeth due to changing properties of diet explains this process, whether it is a phenotype relaxation, or selection for a reduction in size and number of teeth.

Other subsistence patterns that have developed recently in hominin history have also left their mark on parts of our biology. For example, the emergence of agriculture has instigated several distinctive changes that remain with us. The *AMY1* gene, which is responsible for producing salivary amylase, the enzyme that breaks down starch in the mouth by hydrolysing it into more useable sugars, is one such example. Present day humans have several copies of this gene (six is the average), and the more copies an individual has, the more amylase is expressed in their saliva (Bank et al., 1992). *AMY1* copy number variation follows a gradient, such that more copies are present in populations with historically heavy intake of starch, and few copies occur in populations with a low intake of starch (Squires, 1953; Perry et al., 2007; Carpenter et al., 2015; Hardy et al., 2015a). This indicates that some copy number variation evolved recently (during the last 10,000 years) in response to the proliferation of starch in diets from agriculture (Perry et al., 2007). The impact of the lower copy phenotype, a reminder of our hunter-gatherer past, reverberates today as a lower estimated *AMY1* copy-number is linked to obesity and morbidity (Falchi et al., 2014).

As we have seen, our morphology and genome testify to millions of years of changing diets. Dietary adaptations are present from the earliest hominins in early Pleistocene Africa to recent populations in the last 10,000 years. Unsurprisingly, in the case of Neanderthals, subsistence strategies have been a preeminent focus of research. Just as diet was likely the main trait that differentiated early robust and gracile hominins, dietary behaviours are thought to have set Neanderthals apart from other hominins. Neanderthal diet has been suggested as narrow, specialised and profoundly conservative, unlike that of early modern humans, and this dietary niche influenced their range, population history and disappearance.

Neanderthals were closely related to early modern humans, and are even known to have interbred with them, but were distinct in anatomy, ontogeny and techno-cultural expression (Spoor et al., 2003; Smith et al., 2007; Klein, 2009; Gunz et al., 2010; Murray et al., 2015). It is less clear if their diet differed from that of early modern humans. The apparent distinctiveness of Neanderthal resource use has led researchers to link it to their displacement at the end of Middle Palaeolithic. Some consider that Neanderthal diet was reliant on a more restricted range of animal food staples than that of early modern humans (O'Connell, 2006; Stiner, 2013). An inflexible subsistence pattern, due perhaps to cultural or biological factors, may also have burdened Neanderthals with a competitive disadvantage when Upper Palaeolithic modern peoples began to enter Eurasia. Certainly, the more Neanderthals ascended the food chain the more prone they were to experiencing episodes of insufficient food supply. Potentially, this might explain their small isolated populations, their history of regional extinction events and displacement. Unfortunately, it is unclear if their foraging strategies were as inflexible as imagined in this scenario. Furthermore, it is unknown if their economy responded to different ecologies or was static across their range. Understanding their plant use in particular is fundamental because plant use has major implications for their trophic position and the adaptability of their diets. However, because we have limited knowledge on Neanderthal plant use we thus cannot answer these questions. To assess what diet may reveal about this hominin we must first consider Neanderthal origins and history.

2.2 Neanderthals: phylogeography and chronology

2.2.1 Neanderthal origins

Neanderthals evolved from hominins of African origin that entered western Eurasia at some point in the Pleistocene (Hublin, 2009). Revised genetic evidence indicates that the lineage that gave rise to Neanderthals split from the ancestors of early modern humans 550 to 765 ka depending on the pace of the mutation rates (Meyer et al., 2014; Prüfer et al., 2014). A date of about 500 ka would agree with hominin remains found in Europe at this time. Dental morphological evidence poorly converges and it may suggest a split as early as one million years ago (Gomez-Robles et al., 2013). Due to the breadth of these estimated time ranges, and discrepancies between the different lines of evidence, the last common ancestor species is contentious and the geographic setting where it evolved is unclear. Leaving this aside, there appears to be evolutionary continuity in morphology from the hominins found in Europe dated to 500-300 ka (Arago in France, Sima de los Huesos in Iberia, Petralona in Greece and Mauer in Germany) to Neanderthals. Arguably early African fossils have no such continuity to Neanderthals (Bermúdez de Castro, 1997). Palaeoanthropologists can only reliably assign skeletal remains to Neanderthals in the late Middle Pleistocene (230-180 ka) at European sites such as Biache-Saint-Vaast, Fontéchevade, La Chaise Suard, and La Lazaret in France (Churchill, 2014). Most of our knowledge about Neanderthals stems from their later chronological range from 130-30 ka.

2.2.2 Neanderthal range

Neanderthals are known from sites throughout Eurasia, such as Forbes' Quarry, Devil's Tower, Zafarraya, El Sidrón in Iberia; Le Moustier, La Ferrassie, Regourdou, Pech-de-l'Aze, Roc de Marsal and La Chapelle-aux-Saints in France; Neanderthal in Germany; Grotta Guattari in Italy; Krapina and Vindija in Croatia and Kůlna in the Czech Republic; Teshik-Tash in Uzbekistan; Shanidar in Iraq; Amud, Tabun and Kebara in Israel. Although applying a species concept to Neanderthals or any extinct hominin can be difficult and inevitably controversial, the skeletal remains from many of these sites exhibit morphological traits that typify Neanderthals. These sites suggest Neanderthals lived in much of western and central Eurasia. They occupied as far north as the German Coast while in the south their ranged stretched to the Mediterranean rim, the Levant and parts of Iraq (Fig. 1).

Their east to west range stretched from Atlantic Iberia in the west and central Siberia in the east. Skeletal remains show conclusively that they lived as far east as the Altai Mountains in central Asia (Krause et al., 2007). In this span of western Eurasia, there is variation and discontinuity. Occupation of northern areas varied according to glacial cycles (Van Andel et al., 2004). This is evident in the depopulation of northern areas in cold periods of MIS 6 and MIS 4, due to either the volatility of climate or the harshness of the climatic conditions themselves. These regions were subsequently recolonised in warmer phases. Archaeological and mitochondrial DNA evidence shows that this process occurred through a process of incremental local extinctions in northern zones on the onset of cold phases rather than Neanderthals tracking the movement of milder climates south (Hublin, 1998; Roebroeks et al., 2011).

Fig. 1: The area shaded in blue represents the largest known range of Neanderthals based on lithic and skeletal evidence. Krause et al., 2007 modified by Ryulong licenced under CC-BY-SA-3.0 and by author.

2.2.3 Neanderthal disappearance and its implications for dietary ecology

Although Neanderthals survive in part to this day as archaic DNA in the contemporary human genome, Neanderthals are an extinct branch of humanity. The manner of their disappearance has proven to be difficult to resolve. They roamed Eurasia well into the warm MIS 3 Phase (the interplenniglacial), but how late they survived and if their disappearance is a result of the spread of Upper Palaeolithic early modern people is hotly debated (Finlayson, 2008; Pinhasi et al., 2011; Higham et al., 2014). Late Neanderthals may have survived in far-flung pockets of their range, including in Southern Iberia where there are few Aurignacian remains

(Finlayson et al., 2006), and in the Caucasus Mountains (Ovchinnikov et al., 2000). In addition to these postulated refugia, a northern refuge near the Arctic Circle at Byzovaya has been suggested based on Mousterian tools dating to 34-31 ka cal BP (Slimak et al., 2011). This is an exceptionally northern site, outside of the conventional views of their range and it fits poorly with available data (Zwyns et al., 2012). Excavators found no hominin remains, meaning they were unable to clarify if this is a Late Neanderthal occupation. The evidence verifies that Neanderthals were gone across their range by 33 ka cal BP, but they could well have disappeared considerably earlier as dates this late are rare (Galván Santos et al., 2006; Wood et al., 2013a). Many argue that they survived no later than 40 ka cal BP (Pinhasi et al., 2011; Wood et al., 2013b; Higham et al., 2014; Hublin, 2015). One reason why this problem is difficult to resolve is that the period is at the temporal limit of the applicability of radiocarbon dating (Higham, 2011).

Both changes in stone tool technologies and variation within technocomplexes have influenced our interpretation of the disappearance of Neanderthals. Neanderthals developed a stone tool industry called the Mousterian Industrial Complex, characterised by the presence of large, specially prepared cores and specialised flakes often made using the Levallois technique (Klein, 2009). This is the dominant technology in western Eurasia until the arrival of early modern Upper Palaeolithic technocomplexes such as the Aurignacian. In some regions, the archaeological layers containing Neanderthal-associated Mousterian tools are separated from those containing Aurignacian tools by layers containing artefacts from so-called transitional industries. One of the best-studied examples is the Châtelperronian of central France and northern Iberia. Many aspects of the Châtelperronian are characteristically Upper Palaeolithic, leaving some to wonder if it was manufactured by Neanderthals (Ruebens et al., 2015). The Châtelperronian is the only transitional industry with directly associated Neanderthal remains (Hublin et al., 1996), strongly indicating that it was, in fact, created by Neanderthals. Reassessment of Châtelperronian tools appears to suggest that this complex emerged from the local Mousterian (Granger and Lévêque, 1997; Ruebens et al., 2015). This raises the question of Neanderthals groups interacted and exchanged culture with Upper Palaeolithic early modern humans. Resolving this issue is central to understanding the potential intensification of plant use suggested by Châtelperronian grindstones (See 2.5.1). If a process of acculturation occurred, it could have influenced multiple levels of Neanderthal culture including their diets. Debate has centred on whether the Châtelperronian appeared following contact with early moderns. Some have argued that the Châtelperronian was manufactured by

Neanderthals before Upper Palaeolithic modern humans arrived in Europe and that its stratigraphic overlap with moderns at the key Châtelperronian site of Grotte du Renne is a product of sediment disturbances and layer remixing and cannot be reliably interpreted (Zilhão et al., 2006). Indeed, at Grotte du Renne (Arcy-sur-Cure, France), there is evidence of remixing in the sequence (Higham et al., 2010). Reattempts at dating imply that Neanderthals were the makers of the Châtelperronian industry, and the Châtelperronian Neanderthal (Saint-Césaire) post-dated the arrival of early modern humans in western Europe (Hublin et al., 2012). This timing suggests a cultural diffusion from modern to Neanderthal groups.

We can also discern interaction by identifying gene flow between these hominins. Geneticists have pinpointed multiple events of introgression between Neanderthals and early modern humans. This introgression likely occurred in the Near East prior to the split of the ancestors of present day Europeans and Asians (86-37 ka) (Sankararaman et al., 2012). A second event may have occurred, presumably further east concerning the ancestors of present day Asians only (Vernot and Akey, 2015). An early modern human dated to 42-37 ka from Peştera cu Oase, Romania, one of the oldest modern humans found in Europe, showed recent admixture with Neanderthals, with 6–9 % of its genome from a Neanderthal ancestor. The completeness of the archaic DNA in the Oase individual indicates that this cross occurred four to six generations prior. The recent suggested date of this admixture indicates that admixture probably occurred in Europe (Fu et al., 2015).

What this cultural diffusion and population admixture tells us about the Neanderthal subsistence and its ability to adjust to new circumstances is unclear. The presence of two or more types of hominins in Eurasia inevitably led to overlapping territories. Both hominin groups would have sought the same high quality resources, leading to direct competition for the optimal foods. The impact of new hominins on Neanderthals would vary according to how numerous Neanderthals were; a large population could buffer against a large influx of competing hominins. Yet high-quality ancient genomes have revealed that Neanderthal demographic history differs from that of early moderns. Neanderthal genetic history displays a protracted history of small isolated populations and low genetic diversity (Castellano et al., 2014; Prüfer et al., 2014). Small populations could have left them highly vulnerable to even minor competition from early moderns. The inevitable increase in isolation may have reduced their ability to develop resilient subsistence patterns to cope with the arrival of early moderns.

Neanderthal admixture, decline and extinction may imply that their niche was susceptible to competition. It is easy to envisage early moderns who arrived in Europe creating ecological imbalances that disrupted Neanderthal foraging. Given chronological ambiguity, this is not currently detectable. On the other hand, competition may have led to an intensification of Neanderthal subsistence. The cultural diffusion may have led to Neanderthals adopting modern subsistence strategies, but this elucidates little about the dietary niche Neanderthals used for 200,000 years previously. To interpret their long-lived subsistence and dietary regime we must examine their diets using an interpretive framework loaned from ecological theory that allows us to make predictions about their foraging behaviour.

2.3 Applying a framework for studying ancient diets

2.3.1 Human Behavioural Ecology

Knowledge of Palaeolithic human diets is not useful if we have no means of interpreting this information. A theoretical framework that allows us to place dietary choices in a cultural and biological context is needed. Human Behavioural Ecology (HBE) is a useful framework for studying dietary choices and the environmental, biological, and cultural limitations that frame those choices. Behavioural ecology emerged in the 1960s and the 1970s amongst circles of ecological theorists seeking a basis for studying feeding, social, and reproductive behaviours (Bird and O'Connell, 2006). Behavioural ecology spread to researchers interested in human societies, as it allows human behaviour to be interpreted in the rubric of evolutionary ecology theory. Human behavioural ecology posits that individuals tend to adapt to their environment as necessary to maximise their fitness (Mulder and Schacht, 2012). On a daily basis, foragers will consider and weigh decisions on their costs and benefits. If behaviours diverge from this pattern, the possibility of social and cultural factors can be investigated. Behavioural ecologists attribute behavioural diversity to the cumulative impact of the strategies of individuals, the local ecological niches, and the cultural transmissions of information (Smith, 2011; Mulder and Schacht, 2012).

Human behavioural ecology has been widely adopted in anthropology as it offers a framework to generate testable hypotheses about behavioural variation. One group of HBE theories that are popular in studies of human origins are optimal foraging theory and diet breadth models. These models provide a powerful way to assess forager feeding strategies. In a diet breadth framework, researchers predict whether a forager will collect a potential food item that it encounters while foraging (MacArthur and Pianka, 1966). To acquire a resource, the forager must bear both the search costs (e.g. location, hunting and digging) and the handling costs (e.g. preparing and processing), and will minimise these as far as possible although this does not influence a resource's ranking in the diet breadth model. A forager can seek to maximise their efficiency by incorporating the most profitable food items (plant or animal) available and ignoring lower ranked prey (Fig. 2). The inclusion of any given item relies on its ultimate profitability to the forager rather than its abundance (Bird and O'Connell, 2006). Researchers usually assume that foragers assign a rank to a potential food depending on its energy yield minus the cost of food preparation, but foods may also be ranked on other currencies, such as specific macro or micronutrients that are more physiologically important to the individual than total energy (Hockett and Haws, 2005). Food may even be ranked according to individual subjective preferences. The foraging cultures of Alaskan peoples give potential real world examples of non-energetic currencies. Alaskan foragers commonly rob nutritious foods such as sedge corms (Cyperaceae spp.) from rodent caches and nests, which rodents collect for winter food. They compensate the loss of their plunder with fish to sustain the rodent over the winter (Anderson, 1939). A desire to procure vegetable nutrients, rather than energy alone probably explains this behaviour. A low-ranked food item may also be a prey individual that is younger and smaller than normal for the taxon. A food type may be highly ranked, energy-rich and abundant but fast moving, and hard to catch and hence rarely entering diets. Improved technologies can dramatically lower handling costs (Kelly, 1995). For example, nets and weirs majorly abate the search costs for catching river fish, although maintaining nets and weirs is a substantial long-term cost and a constraint on mobility. For this reason, recognising technological change is important for deciphering foraging choices.

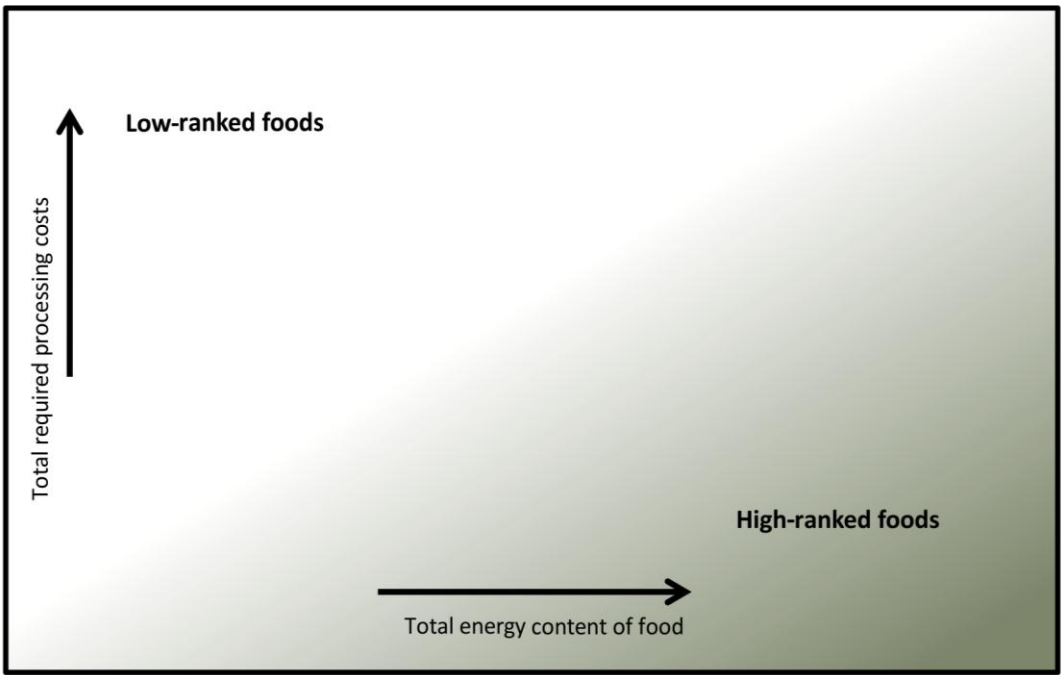

Fig. 2: Conceptional illustration of a diet breadth model that uses energy. Behavioural ecologists rank foods on their energy (or other nutritional metric) return net of the processing costs expended to obtain energy. Ranking in diet breadth models ignores prey abundance and prey search costs.

Given current data on handling costs, diet breadth models predict that due to the differences in energy yield, animal foods (especially meat and fat from medium and large ungulates) and honey are high-ranked, in contrast to the majority of plant foods (Table 1). However, plant foods are usually a more abundant staple and their collection is far more reliable than most types of hunting. Nevertheless, because of their laborious collection and handling most plants provide fewer calories (Kelly, 1995; Kuhn and Stiner, 2006), and are less preferred. Ethnographic studies of food preferences confirm this trend (Berbesque and Marlowe, 2009). Although recent foragers prefer animal foods, plant foods are still a ubiquitous feature of their diets. This is readily explained by the fact that the quest for food relies on reducing food supply volatility, rather than always attempting to maximise the amount of food one takes in (Winterhalder, 1986). Plants are usually more abundant and occur in large patches, they are more easily and reliably located than game. This is important because forager mobility is limited.

A lesson in the value of these diet breadth models in interpreting food choice may be found in the classic ethnography of the Aché of eastern Paraguay (Hawkes et al., 1982). The Aché hunt peccary, deer, and monkey, and they gather oranges, palm hearts, palm fibre and larvae in palms as well as honey. Hunting offers unreliable returns yet the Aché are predominantly hunters. Game provide about ~78 % of dietary energy while plants provide only ~12 % (Gurven et al., 2001). Plants and fish available to the Aché offer more abundant, more reliable, and far more readily located foods. Aché hunting game will stop and collect honey, grubs, and the most highly ranked plants they pass such as oranges. Yet they often ignore other plant foods like palm fibre even though these are regularly consumed when little else is available. The Aché provide several lessons for studying ancient hominin food provision. Examination of energy returns reveals that hunting is typically the optimal strategy, although unreliable, hunting offers the highest overall returns. Although plants provide less energy (Kelly, 1995), they are crucial and ensure survival in periods of shortage. It also is a reminder of the uneven distribution of resources in the landscape (Hawkes et al., 1982). While this patchiness is unaccounted for in diet breadth models it is incorporated into other theories such as the patch choice model and the marginal value theorem. Although these theories are difficult to apply to Palaeolithic societies, they further illustrate why plants are collected for subsistence. The patchy nature of food distribution combined with labour specialisation favours the common use of both high- and low-ranked foods. This strategy is true in most societies worldwide.

Recent foragers exploit variations in the reliability and abundance of plant and animal foods with highly efficient systems of labour specialisation, usually according to sex. Generally, in recent forager societies females collect most plant foods while males tend to hunt, especially when the quarry is large and dangerous (Kelly, 1995).

This sex specialization pattern is true in almost all reported recent forager societies, except the Agta of the Philippines (Goodman et al., 1985). One advantage of this system is that it accommodates the difficulties that a highly mobile hunting lifestyle brings to aspects of reproduction such as breast-feeding. The gathering of plants and immobile animal foods like shellfish is more conductive than hunting to the feeding demands of infants, and reduces the risk starvation because gathering yields nutrients even when hunting fails (Brown, 1970). Moreover, hunting large game may have been useful to males as a method of building social status by signalling fitness to the group, this is known as "costly signalling" (Hawkes and Bird, 2002). A division of labour between hunting and foraging thus optimises the high-energy returns of meat and the reliability of plant collection. The risk of a diet that eschews plant food and depends on animal food is observed in the early records of arctic foraging peoples. Little plant nutrition was available to arctic foragers and this coupled with extreme seasonality meant that stretches of hunger and even devastating starvation were a regular feature of life (Ackerknecht, 1948; Young, 1996). Records from isolated communities in eastern Greenland show that as many as 15 % of the population died of starvation between 1881 and 1883 (Holm, 1911).

2.3.2 The subsistence trajectory of Palaeolithic societies

Diet breadth models provide a starting point for exploring subsistence choices of past groups. They have formed the dominant paradigm to explain Palaeolithic subsistence. Prehistorians have invoked behavioural ecology to argue that there is a landmark point in hominin dietary history at which foragers switched prey in a transition termed the "Broad Spectrum Revolution" in the terminal Pleistocene (Binford, 1968; Flannery, 1969; Zeder, 2012). Hunters of medium and large game began to heavily rely on plants, fish, and fowl due to climatic and demographic pressures. In addition, broad spectrum foragers existed in higher densities and typically spent a high proportion of time processing food. An increasing use of plants was believed to induce a local lowering of the overall human trophic level. This changing strategy led to an increase in dietary breadth, and ushered in the first experiments in providing food through agriculture and pastoralism.

Zooarchaeologists have since argued that the process of prey switching and diet broadening occurred far earlier, at the start of the Upper Palaeolithic. Stiner and colleagues (1999) noted an increased frequency of small fast-moving prey such as hares, which yield lean meat and are therefore low-ranked, at the start of the Upper Palaeolithic. This change came at the same time as a reduction of body size in hunted tortoises, which reflected the increased hunting pressure on this preferred, easy-to-acquire prey. This contrasted with the pattern of zooarchaeological remains seen among Middle Palaeolithic Neanderthals in the same geographic regions, which exhibited a rigid subsistence strategy centred on hunting the most high-ranked of resources prime-aged medium and large game (Stiner, 1994, 2013; Stiner and Kuhn, 2006).

Stiner (2013) argued the Neanderthal dietary niche changed little in the hundreds of thousands of years they occupied Eurasia and that it is far easier to find differences between the Middle Palaeolithic and other periods than within it. This largely static Middle Palaeolithic foraging niche, narrow and inflexible in most regions, placed a ceiling on the carrying capacity and ensured a very low population density. In this perspective, as Upper Palaeolithic modern humans entered Eurasia their broader niche meant they would inevitably displace Neanderthals across all of their range through a gradual process of competitive exclusion (Stiner et al., 2000; Hockett and Haws, 2003; Kuhn and Stiner, 2006; O'Connell, 2006; Stiner and Kuhn, 2009).

Comparing plant consumption with the above animal food models is challenging due to the visibility bias in favour of animal foods. Plant remains are unlikely to survive on most Palaeolithic sites, thwarting attempts to extrapolate plant use with conventional approaches, despite being potentially essential for nutrition (see below) (Speth, 2010). The difficulties faced are exacerbated if data are viewed in isolation. The dissertation predicts that the contribution of plant foods to Palaeolithic diets breadth is masked by this taphonomic bias. It hypothesises that it may be possible to examine the plant contribution to dietary breadth by quantifying the variety of plant foods represented in dental calculus.

However, before this can take place, a synthesis is needed that re-evaluates the evidence of plant use in botanical, artefact, genetic and osteological studies to contextualise this information. This process requires that we first establish what plant food may have been available to Neanderthals and which were likely to have been most important from a behavioural ecology perspective.

2.3.3 A behavioural ecology model for Eurasian environments

Although edible energy-rich plants were present throughout western Eurasia (Sandgathe and Hayden, 2003; Hardy, 2010), there is a dearth of data on their availability. We can mathematically predict the total biomass of plants in Pleistocene environments, but not the total biomass of edible plants. Ethnographic data gives us the option of modelling Neanderthal plant use if they resembled recent foragers and if Pleistocene environments were similar. Assuming Neanderthals fell within the ecological gradient present in the economies of recent northern foraging people, plants would have been a significant part of their diet. Churchill (2014) used net primary productivity and effective temperature to predict Neanderthal dependence on plant consumption based on recent foragers. This model estimates that plant intake represented 11-25 % of diet in the coniferous forests north of the Alps, rising to 36-43 % in the temperate forest of the last interglacial MIS 5e.

Table 1: Energy yields of various food classes consumed by recent foragers. Reprinted from 'What's a Mother to Do? The Division of Labor among Neandertals and Modern Humans in Eurasia' by Kuhn and Stiner (2006).

	N cases	kJ/hr			kJ/kg	
		Mean	Min.	Max.	Mean	Sd
Large game	4	63,398[a]	36,000	75,115	6,980[b]	1,383
Small game	14	16,034[a]	1,672	56,317	6,980[b]	1,383
Reptiles	3	15,850[a]	17,556	12,435	4,489[b]	715
Birds	3	4,472[a]	961	8,255		
Roots and tubers	14	6,120[a]	418	26,133	2,926[c]	1,680
Roots and tubers	9	10412[a]	3,695	23,333	2,926[c]	1,680
Roots and tubers	13	1882[d]	1,045	2,300	3,136[d]	2,338
Seeds and nuts	34	3,520[a]	380	18,538	13,188[c]	9,334
Seeds and nuts	9	6,508[e]	1,203	24,933	13,188[c]	9,334
Seeds and nuts	6				19372[d]	6,250
Foliage					1,250[c]	819
Foliage	3				1,534[d]	186
Fruits					2,403[c]	1,463

[a] Data from Kelly (1995, table 3.3).

[b] Data from Hawkes et al.(1982), Hurtado and Hill (1987).

[c] Data from Pennington (1989)

[d] Data from Wiessner (2003 and personal communication); cases are from Nyae Nyae area minus those where elephant damage was severe for tubers.

[e] Data from Wright (1994, table 2).

There is also considerable ambiguity about candidate vegetal food staples and the types of low- and high-rank foods that we may expect in Pleistocene Eurasia. The diet-breadth model stipulates that abundance is no predictor of a resources' value, meaning that botanical surveys of species frequency are inadequate for extrapolating staples. There are few studies detailing the returns and costs of acquiring Eurasian plant foods that would allow us to develop a detailed set of predictions (Martinoli, 2005). Summaries of ethnographic data mostly from Great Basin foragers indicate that of all classes of plant foods, two categories offer the highest net energy per hour: seeds/nuts, and underground storage organs (Table 1). Underground storage organ returns exceed those from seeds/nuts, but they still return less energy than nearly all classes of animal foods (Kuhn and Stiner, 2006). Researchers have referred to USOs as a mainly African resource (Kuhn and Stiner, 2006), but this is based on anecdotal evidence and may not be reliable. Generalisations are inadequate for reconstructing Neanderthal dietary ecology because understanding plant use requires systematic data on plant and animal food variables. We know that in some cases prodigious amounts of USOs were available in Eurasia. For example, reedmace (*Typha* spp.) provides extensive and dense concentrations of edible USO biomass in marshes, river and lake shores (Morton, 1975). Unfortunately, detailed ethnographic data of foragers that specialised in wetlands that we could use to model Pleistocene wetland foraging is unavailable, because such societies disappeared before they were studied in detail.

Although there is little information on how the edible plant food biomass varied in different Pleistocene habitats, we may presume that the availability of energy-rich plant foods was superior in southern regions. Open Mediterranean woodland and wetlands would have supported a greater diversity of plant foods than cold steppe or coniferous forest (Kelly, 1995). In northern areas, although winters were intensely cold, strong winds often blew away snow, thus exposing ground-based resources such as edible tubers (Guthrie, 2001). During glacial phases moisture loving edible plants were drastically limited by the intense aridity but edible plants tolerant of dry conditions may have been abundant in the rich often-alkaline steppe soils.

The use of plant foods by recent foragers makes it possible to suggest possible plant food staples. Diet breadth model criteria (Fig. 2) can indicate whether a food was high- or low-ranked (Haws, 2004). In Mediterranean woodlands, the high-ranked plants probably constituted nuts like pistachio (*Pistacia* sp.), olive (*Olea* spp.), chestnut (*Castanea sativa*), some aquatic plants such as water chestnut (*Trapa natans*) and certain underground storage organs including as burdock (*Arctium lappa*). Mid- or low-ranked foods likely included seeds of grasses (Poaceae), Madroños (*Arbutus* sp.), gum rockrose (*Cistus ladanifer*) seeds, acorns (*Quercus* spp.), legumes, and various woodland, coastal, and wetland underground storage organs (though some of these foods may have been relatively high-rank) such as honesty (*Lunaria annua*). In northern environments, high-ranked foods may have included reedmace, hazelnut (*Corylus avellana*), and sea kale (*Crambe martima*). Lower ranked foods probably included various underground storage organs like pignut (*Conopodium majus*), Asteraceae taproots such as dandelion (*Taraxacum)*, sea holly (*Eryngium maritimum*), silverweed (*Argentina anserine*), and lilies (*Lilium* spp.), as well as seeds such as grass grains, and acorns and perhaps pine bark (*Pinus* spp.). Most of these foods are relatively energy rich, though many have some processing costs. These species are far from a complete list of edible Eurasian plants but they are likely particularly important to Eurasia foragers. Across many of the environments Neanderthals occupied, a range of other less energy-dense foods were available, including mushrooms, leafy tissues, stems, drupes, berries, and seaweeds. In addition to the total energy content, food macro- and micro-nutritional qualities influence forager selection (Hill, 1988). Unfortunately, micronutrient data from wild plants are extremely rarely compiled and most nutritional data that are available only covers a fraction of the whole nutritional spectrum So my project focuses on total energy and macro-nutrients rather than or micro-nutrients.

2.4 The nutritional requirements of Neanderthals

2.4.1 Neanderthal energetics

Distinctive cranial and postcranial morphology distinguishes Neanderthals from other hominins (Tattersall and Schwartz, 1999). Neanderthals' anatomy and inability to reduce the cost of mobility with technology presented energetic challenges, which may have limited their diet (Verpoorte, 2006; Churchill, 2014). Overall body proportions show the most resemblance to present-day cold-adapted populations (Trinkaus, 1981). Scientists interested in Neanderthal energetics have estimated their body mass range in order to calculate their energetic requirements, and though these estimates vary depending on the technique and skeletons used, all versions indicate that the body mass of Neanderthals was very high. Although Neanderthals stood at a comparable height to recent Arctic forgers, they could have weighed considerably more (Churchill, 2014). Well-developed muscular attachments demonstrate that they had heightened muscularity (Churchill and Rhodes, 2006). Overall, this indicates Neanderthals required increased amounts of energy compared with other hominins. Estimates of basic daily energy expenditure vary depending on supposed physical activity level but figures suggest a metabolic requirement far above the average of any human forager group (Sorensen and Leonard, 2001; Snodgrass and Leonard, 2009). To fulfil their energy requirements an ample energetic return from foraging would have been critical (Sorensen and Leonard, 2001). Recent studies have re-examined Neanderthal energy expenditure and suggest that although their locomotion required more energy than early modern humans, the difference is less than previously thought (Hora and Sladek, 2014). Heyes and MacDonald (2015) have pointed out that the error range in comparisons between European Neanderthals and Upper Palaeolithic early modern humans is too great to identify any differences in body mass. However, recent foragers differ substantially anatomically from early moderns. Our energetic model uses recent foragers rather than Upper Palaeolithic early moderns as an energetic yardstick.

Animal foods, such as muscle, fat, marrow, and organs, were the most calorie-rich foods available to Pleistocene foragers. However, there are physiological limits to animal food diets. If an animal-based diet contains insufficient fat, it can lead to protein poisoning. Consuming protein beyond a safe threshold leads to a progressive onset of nausea, wasting and eventual death (Speth, 2010; Butterworth et al., 2016). Unfortunately, many crucial details of this condition are unknown but nutritionists have gathered some information from ethnographic sources and small-scale trials (Stefansson, 1956; Speth, 2010). The notes of explorers and athletic studies show that people unaccustomed to a high protein intake may rapidly adjust in weeks to high protein ingestion but a protein ceiling remains (Phinney, 1995; Speth, 2010). Extant hunter-gatherers regularly consume vast amounts of lean protein when gorging on meat after kills. However, health records suggest that hunters who gorge in the dry season when game is thin lose weight (Speth, 2010). Recent Arctic foragers knew the dangers of consuming excessive lean meat and referred to protein poisoning as rabbit malaise (Stefansson, 1956). Although protein may have formed a large part of Neanderthals' dietary intake, a diet of mostly protein is improbable (Speth, 2010). Nutritionists argue that recent foragers who consume mostly animal foods overcome these problems by maximising their intake of fat. Neanderthal hunters must have relied especially on fatty meat to avoid this problem. Carbohydrates in mammal liver (absent elsewhere due to the effects of rigor mortis), shellfish and plants could have also played a role (Cordain et al., 2000).

The potential dangers of specialised animal food diets are highlighted in the case of pregnancy. Hockett (2012) has argued that given the formidable estimated daily energy expenditure, a diet consisting of only medium- and large-game would kill a pregnant Neanderthal due to excess protein, vitamin A, niacin, iron, zinc, and selenium in addition to a major calcium deficiency. This model attempted to take into account a variety of different levels of activity and thermoregulation, but in all cases, Hockett argued that a terrestrial animal-food-only diet was toxic to pregnant females.

2.4.2 Macronutrients provisioning

Along with total protein intake, specific amino acids must be consumed for maintaining health and ensuring correct development. These nutrients are an imperative for all mammals and Neanderthals must have acquired them. The body requires twenty amino acids and about nine must be directly sourced from food. A meat-rich diet would have adequately provisioned Neanderthals with these nine amino acids (Churchill, 2014). In contrast, sourcing essential fatty acids may have been more difficult. The essential long-chain polyunsaturated fatty acids docosahexaenoic acid (DHA) and arachidonic acid (AA) are required for brain growth. Obtaining sufficient DHA would have been particularly crucial during pregnancy and lactation (Crawford et al., 2008). The body may synthesise them from linoleic acid (LA) and α-linolenic acid (LNA), but this is less efficient than acquiring them directly. Consumers can derive LA and LNA from some domesticated plants (e.g. flaxseed), but it is unclear if they occur abundantly in wild plant foods (Simopoulos, 2004). The highest DHA concentrations are found in marine foods. Foragers could also access this fatty acid through terrestrial mammals, but only in far lower concentrations, and only in certain tissues like the brain. AA is also present in terrestrial animals, especially in the viscera, but there are no highly concentrated sources of AA in large ungulates. Thus, AA deficiencies may have caused problems. Neanderthals, especially when pregnant, may have required additional AA or DHA. Supplementary intake of marine fish and mammal brains would be more important if they consumed a large amount of plant material low in these fatty acids. Neanderthals may have targeted smaller prey for DHA, as the ratio of brain size to body mass is larger in smaller mammals (Crawford et al., 2008). This selective feeding approach would also alleviate the particular risk of protein poisoning in late winter and early spring when prey had expended their stores of fat over the winter (Speth, 2010).

We can see that there is a need for a convincing energetic model for Neanderthals. It is possible that Neanderthals had adaptations that regulated their essential nutritional demands to what Pleistocene Eurasia could provide, but this is conjectural. Yet we can deduce without doubt that the large build, muscularity and large brain that Neanderthals evolved presented energetic and nutritional challenges although they must have offered some selective advantages. Neanderthal nutritional demands for fatty acids were the cost of their large and metabolically expensive brains. This has led Churchill (2014) to argue that the extent to which Neanderthals relied on plants was constrained by the sheer volume of meat they needed to obtain essential fatty acids in the absence of fish and shellfish consumption. This hypothesis is based on incomplete data of the nutritional opportunities of Pleistocene plant foods. Neanderthals may have alleviated nutritional deficiencies with selective use of certain resources including plants rich in fatty acids, mammal brains, and marine and freshwater fish.

2.5 Extant biological and technological traces of diet

Neanderthals were among the first hominins to spread into cold temperate and glacial environments (Hublin and Roebroeks, 2009), and by studying their dietary ecology, we may gain a better understanding of the strategies and adaptations that allowed them to thrive in these inimical environments. We may also find better explanations of how this close relative survived through 200,000 years of shifting climate, changing ecologies, and carnivore competition only to abruptly disappear as early modern humans entered Europe. The gradual expansion of research using macromammal remains allowed substantial insights into Neanderthal meat consumption, hunting techniques, and social cooperation (Stiner and Kuhn, 2006; Stiner, 2013; Churchill, 2014). There has been, however, limited ability to examine their complete dietary ecology. The recent advent and maturation of new approaches in archaeological sciences such as dental wear, isotope, biomarker, and dental calculus analyses has allowed considerable strides in building a more complete view of their dietary ecology. Although these advances have answered some questions, they have raised others. To explain diet, it is necessary to review and synthesise this evidence.

2.5.1 The implications Neanderthal technology for subsistence

Hunter-gatherer toolkits can have specialised functions for the collection of specific resources. Specialised toolkits can reveal resource use if the functional design can be identified by archaeologists. For instance, later Levantine foragers produced knapped sickle blades that reflect widespread harvesting of wild grasses (Bar-Yosef, 1998; Goodale et al., 2010). For these reasons, it is important to review Neanderthal technology and evaluate if it suggests gathering of plants for food.

Recent hunter-gatherers give us an approximate picture of the tools that Neanderthals may have used to gather plants. Implements such as stout digging sticks, folded bark containers, or seed and fruit beaters may have been used, but these organic items will seldom survive the vast periods of time that has passed. A handful of wooden implements have survived in special circumstances from 400-125 ka in present-day Germany and England (Thieme, 2000). Mostly these are interpreted as spears due to their length (1.8- 2.5 m) and pointed tips, however, digging sticks known from the ethnographic record reach up to 2 m (Nilles, 1942; Boesch, 2012). Thus, such wooden tools could plausibly have been used as digging sticks, but they would be unusually long examples. Worked sticks resembling digging sticks in length have also been found at Schöningen (Schoch et al., 2015). Foraging tools are inclined to be made, used, and disposed over a short period, leaving few diagnostic markings or use-wear, and thus identification would be difficult. Furthermore, many of the plant collection tools in the repertoire of recent foragers are highly multifunctional, for example, foragers can use the same sticks for prying off edible inner tree bark as they use for dispatching game.

Unlike organic tools, stone tools are regularly preserved in the archaeological record. Knapped stone tools would have been extremely useful for preparing plant foods and shaping wood, despite being usually associated with animal butchery (Langejans, 2012; Hardy et al., 2013; Wadley and Langejans, 2014). Some Middle Palaeolithic flaked stone tools do preserve evidence of plant processing (Hardy et al., 2001; Hardy and Moncel, 2011). An alternative proxy line of evidence may be found in the stone technology used to process food (milling, scraping, and pounding); for instance, ground stone artefacts may be specialised implements for processing grass seed (Wright, 1994; de Beaune, 2004; Dubreuil and Nadel, 2015). Like other tools, they have a generalised function and are used for preparing minerals as well as processing plants. Ground stone tools for macerating and pulverising have been widely identified in early Upper Palaeolithic occupations although unequivocal verification that these ground stones were specifically used for plant processing is less forthcoming. Ground stones are known in African Middle Stone Age contexts (McBrearty and Brooks, 2000), but their prevalence is poorly known (Stiner, 2013). In contrast, ground stones are rarely present in Middle Palaeolithic deposits, but they are frequently found in Châtelperronian contexts (Straus, 1992; de Beaune, 1993; Churchill, 2014; Power et al., 2015a). One potential Middle Palaeolithic case was found associated with pine nuts at Gorham's Cave in Gibraltar (Barton et al., 1999). This has been interpreted as a tentative nutcracker. Middle Palaeolithic hominins may have used modest pieces of naturally shaped stone (manuports) to grind seeds and nuts, perhaps compatible with their highly mobile lifestyle. Likewise, the cobble hammers they used for knapping may have been serviceable for plant processing. Archaeologists might have overlooked such simple toolkits that used unmodified stones, while the same is not true if they used heavily modified specialised technology, like in the Epipalaeolithic of the eastern Mediterranean. In sum, we cannot infer plant use from their technology. Neanderthals could have widely used grinding stones, but they did not invest in unperishable specialised processing tools. This appears to be a reliable difference from the technology employed by early modern humans.

2.5.2 The genetic evidence of diet

The unfolding of genomic data from both living and fossil hominin specimens has opened a door to a vast array of data on nearly all aspects of hominin biology (Stoneking and Krause, 2011). With these data, we can test hypotheses about how hominins biologically adapted to varying diets. The process of piecing together Neanderthal genetic history has been an incremental undertaking but some insights into selection relating to diet are already available (Perry et al., 2007). Comparison of the genomes of contemporary humans, chimpanzees, Neanderthals and Denisovans has shown evidence that Neanderthals, Denisovans and present day humans all lost a masticatory myosin gene (*MYH16*) that helps develop powerful masticatory capabilities in chimpanzees. Changes in hominin social structure may have contributed to this but to a large extent this gracilisation is linked to the gradual adoption of a more energy-dense, softer diet, potentially around ~2 ma (Perry et al., 2015).

Research has also found that Neanderthals carried activated and deactivated variants of a gene for a bitter taste receptor- *TAS2R38* (Lalueza-Fox et al., 2009). *TAS2R38* detects a compound called PTC (phenylthiocarbamide). PTC does not occur in plants, but sensitivity to PTC reflects ability to experience bitter tastes in certain plants such as members of the cabbage (*Brassica*) genus (Kaplan et al., 1976). It seems Neanderthals may have experienced variable sensitivity to PTC, as do early modern humans. Early modern humans, Neanderthals and Denisovans have lost two genes rating to bitter taste (*TAS2R62* and *TAS2R64*) that are still operative in chimpanzees. Although in contemporary humans factors such as personal preference contribute to use of the plants (Niewind et al., 1988) this gene loss means that they experienced this taste variably as people do today (Perry et al., 2015). Some report that contemporary humans with the activated *TAS2R38* gene eat fewer plants, but bitter taste receptors may serve to protect against toxins and be quite useful for identifying plants that are safe to eat. It should be noted that in contemporary humans non-genetic factors such as personal preference contribute to use of the plants (Niewind et al., 1988). Low sensitivity to bitterness indicates that Neanderthals shared specific adaptations with early modern humans associated to the consumption of particular plant foods and more energy-dense diets. Yet these pseudogenising (gene loss) mutations occurred long before the divergence between Neanderthals and early modern humans, and they reflect selection in populations that predate Neanderthals (Perry et al., 2015). Though we currently have no way of knowing if these adaptations were of dietary importance for Neanderthals, they probably were not.

Not all selection events that relate to diet predate the Neanderthal divergence from African populations. Like chimpanzees and humans, Neanderthals possess the salivary amylase gene (*AMY1*) enabling them to break down starch into more useable sugars in the mouth (Perry et al., 2015). Neanderthals, like chimpanzees and Denisovans, carried only one to two copies of the salivary amylase gene. However, the contemporary human lineage carries a higher number of copies depending on the population. Contemporary humans on an average have about six copies of the *AMY1* gene. This difference is thought to have emerged in Africa during the past 200,000 years, long after Neanderthals diverged roughly 600,000 years ago (Perry et al., 2015). We do not know why copies of *AMY1* were selected, since most starch digestion occurs in the gastrointestinal tract from pancreatic amylase activity (Lee et al., 2013). Some have proposed that oral starch digestion may have been lifesaving in infants, which have minimal pancreatic activity (Butterworth et al., 2011; Hardy et al., 2015a). Extra copies may have arisen to boost protection against death from diarrheal and intestinal disease in groups heavily reliant on starchy plant foods (Perry et al., 2007). The high number of copies of *AMY1* probably reflects the importance of starchy plant foods to early African humans. Some might argue that the fact that Neanderthals had few *AMY1* copies implies a low use of plants. However Neanderthal *AMY1* copy number reveals limited insight into their total intake of plants, because starch is absent in many nutritious plant foods. New World primates lack *AMY1* despite being obligate plant eaters (Perry et al., 2007).

2.5.3 Zoological traces of diet

The vast bulk of data concerning Middle Palaeolithic foraging stems from skeletal remains recovered from archaeological sites. Faunal remains are far the most numerous dataset available to researchers, even though many faunal assemblages are natural accumulations and not a product of hominin activity. This is due to fact that the karstic caves that dominate Palaeolithic archaeological research in Europe provide good environments for the preservation of skeletal remains, and act as landscape bone traps. Anthropogenic macromammal skeletal remains have been used to target a wealth of questions on meat provisioning capabilities, dietary breadth, and intensity of resource use. Faunal assemblages are frequently palimpsests, a sum of many unrelated episodes, such as hunting, scavenging, natural death jumbled by unpredictable sedimentary processes, which leaves a complicated formation history (Lyman, 2003). Despite the difficulties in reconstructing economic strategies from skeletal remains, zooarchaeologists have accumulated much information about Neanderthal- animal interactions. Zooarchaeologists once argued that Neanderthals were primarily scavengers, due to presumed cognitive or technical limitations (Binford 1985) but zooarchaeological data have verified that Neanderthals were capable hunters who exploited a variety of game (e.g. Speth and Tchernov, 2001). Neanderthal hunting technology is distinct and appears to have centred on handheld hafted and unhafted spears used mainly for thrusting (Villa and Soriano, 2010). Specialists argue that Neanderthals' close range hunting technology and susceptibility to carnivores meant they may have depended on closed forests, ecotones, or brush-grass mosaic habitats for much of their kills, although they clearly ventured into open country to hunt at times (Churchill, 2014).

Neanderthal hunting strategies throughout their range focused on a handful of key mammals, typically prime-aged artiodactyls. However, Neanderthals were capable of exploiting most of the herbivorous taxa that they encountered (Churchill, 2014). The largest game available on the landscape is rare in Neanderthal sites, but there are traces of the consumption of some of these fauna including mammoths (e.g. Germonpré et al., 2014). They also hunted large, dangerous predators such as bears, leopards, and cave lions (e.g. Valensi and Psathi, 2004; Blasco et al., 2010). However, prey exploitation was heterogeneous across their range. Resource choice followed ecological gradients of the period. On the European Plain fauna that lived in open or mixed areas such as horse (*Equus* sp.), woolly rhino (*Coelodonta* sp.), ibex (*Capra* sp.), red deer (*Cervus elaphus*) and to a lesser extent reindeer (*Rangifer* sp.) were preferred (Patou-Mathis, 2000). Yet in Italy, Neanderthals seem to have favoured red, fallow (*Dama dama*) and roe deer (*Capreolus* sp.), suggesting a preference for closed habitats (Stiner, 1994). In Iberia, a pattern of red deer, horse, ibex, wild boar (*Sus* sp.) and bovine exploitation has emerged in the zoological reports although considerable variability is present. While these data seem to indicate a relatively static hunting pattern focused on ungulates, there are a few exceptional sites where other distinct varieties of animals were consumed. Some sites (Figueira Brava, Vanguard, Gorham's Caves and Grotta di Sant' Agostino) in Iberia and Italy have evidence for consumption of monk and ring seals (*Monachus monachus* and *Pusa hispida*), porpoises (*Phocoena phocoena*) and dolphins (*Tursiops truncate* and *Delphinus delphis)* (Antunes and Santinho-Cunha, 1992; Stiner, 1994; Stringer et al., 2008). Other sites (Bajondillo Cave, Bolomor Cave and Hayonim Cave) on the Mediterranean rim have abundant small game components (Cortés-Sánchez et al., 2011; Blasco and

Fernández Peris, 2012). Fauna analysts have studied Châtelperronian large-game fauna assemblages left by late Neanderthals and reported few differences from Aurignacian assemblages (Grayson and Delpech, 2008), but large game hunting practises are generally similar between the Middle and the Upper Palaeolithic (Stiner, 2013).

The role played by small animal prey is far more useful for distinguishing Neanderthal and early modern human subsistence, but it remains poorly understood (Fiorenza et al., 2015). The relative lack of information about small game is due in part to taphonomic problems. Small animals are less likely to be preserved, recovered, and identified (Yellen, 1991a; b). Foragers may consume small game in the field rather than bringing them to camp. Small game remains are harder to conclusively associate with hominin use, because they may be deposited in an archaeological site by carnivores or birds of prey, or the small animals may have simply lived, and died, in the site. Furthermore, small game can require less butchering to process and consume making them even harder to associate with hominin activity (Brown et al., 2011). Nonetheless, there is sufficient data to demonstrate that Neanderthals living in southern regions targeted some species of small game, including rabbits, birds and tortoise (Stiner et al., 2000; Blasco et al., 2013; Salazar-García et al., 2013). In some cases, such as Grotta dei Moscerini in central Italy and Hayonim Cave in Israel, small game like shellfish, tortoises, lizards, and ostrich eggs compromise 45 % or more of the faunal assemblage (Stiner, 1994). In central and northern Europe, Neanderthals also consumed small game during the MIS 5e interglacial. In Taubach (Germany) and Vindija (Croatia), there are many cut-marked beaver (*Castor fiber*) bones (Gaudzinski-Windheuser and Roebroeks, 2011).

Fish bones have been found in several sites in western Italy, France, and southern Iberia (Fiore et al., 2004; Fiorenza et al., 2015), as well as Vindija Cave in Croatia (Paunović and Smith, 2002) and at Raj Cave in Poland. Like small terrestrial game and shellfish, fish remains are rare in Mousterian levels of Palaeolithic sites. Even where fish remains are present, they are less frequent than in the contemporaneous Middle Stone Age sites in Africa (Klein and Steele, 2008), but this disparity may be related to sea level changes, as many western Middle Palaeolithic coastlines are currently under water and thus not available for study. In addition, fish remains are even less likely to be preserved than terrestrial small game (Szpak, 2011). A variety of shellfish species were found in Middle Palaeolithic sites in Iberia, Italy and Greece, but they are very rare (Stiner, 1994; Klein and Steele, 2008).

The use of small game and aquatic resources directly relates to dietary breadth. Zooarchaeologists usually consider small game a low-ranked prey item, because the costs are high relative to the amount of food each prey item provides. For example, a hunter may expend considerable energy to bag a fast-moving and agile hare with limited energetic returns, unless they use technology to assist the process. Dependence on low-ranked prey is often linked to the declining supply of high-ranked large and medium-sized game, population growth, and technological investments in energy capture (Stiner et al., 2000). However, regarding all small game as low-ranked prey is overly simplistic (Fiorenza et al., 2015). Some small prey, such as tortoises and eggs, may yield high returns with little foraging costs. In other cases, small prey may be low-return but may offer specific macronutrients (e.g. DHA in marine foods or small mammal brain) more important than total energy (Kelly, 1995; Winterhalder and Smith, 2000; Haws and Hockett, 2004). For these reasons, zooarchaeologists have attempted higher resolution approaches including sub classifying small game by ease of capture and species diversity (Stiner, 2001). These dietary breadth metrics have been used to argue that Neanderthals very rarely captured low-ranked small game, in contrast to recent hunter-gatherers, some Upper Palaeolithic foragers, and possibly some Middle Stone Age foragers (Stiner, 2013). However, there is still some apparent variability in Neanderthal behaviour. At Kebara Cave, Neanderthals increased their reliance on low-rank juvenile gazelle and fallow deer (low-rank due to their small body size and reduced adipose tissue), while the relative proportion of high-ranked aurochs, red deer, and boar decreased from 50 ka onwards. In this case, it appears that Neanderthals depleted large game supplies, and were forced to adapt through prey switching (Speth and Clark, 2006).

In sum, Neanderthals appear to have been capable hunters who favoured hunting medium and large game. The absence of small game, fish, and shellfish in their range in central and northern western Eurasia is due to a combination of bias in the archaeological signal and intentional hunting strategy. Although the Middle Palaeolithic predation niche varied considerably through time and space, the general pattern of Neanderthals as medium- and large-game hunters is to some extent correct. However, in some cases they were also consumers of other foods such as small mammals, fish, shellfish, bird eggs, lizards, and scavenged meat. Unfortunately, researchers have yet to describe full geographic and temporal variation in small-game procurement (Fiorenza et al., 2015). Nevertheless, the majority of studied faunal assemblages indicate most Neanderthals appear to have engaged in narrow spectrum foraging for most Neanderthal populations, with some evidence for increased dietary breadth beginning about 50 ka (Speth and Clark, 2006; Stiner, 2013).

2.5.4 Indications of diet from macrobotanical plant remains

Evidence for the consumption of plant foods is sparse across the Neanderthal range in part because, unlike bones, most plant remains require a specific set of exceptional circumstances to preserve in archaeological deposits. In the western Eurasian context, this is typically carbonisation, though desiccation and waterlogging are also possible (Van der Veen, 2007). Carbonisation requires that the food plants are exposed to fire and typically best preserves seeds and nuts that benefit from cooking. The record of charred remains is biased in other ways too, because foragers frequently consume plant foods as they are collected, before returning to camp and encountering fire (Marlowe, 2010). In addition, macrobotanical assemblages poorly preserve tissue of some vegetal resources such as underground storage organs, leafy plant parts and oily plant foods (Pennington and Weber, 2004), rendering these foods largely invisible to archaeologists. Plant remains cannot be representatively recovered or even detected onsite without specialised archaeobotanical sampling, which the archaeobotanical community only commenced in the 1960s. Therefore, and unlike studies of fauna, literature so scarcely discussed botanical remains that some archaeologists may have avoided monitoring for them even after the techniques were available. In addition, even if plant remains are found, they may simply reflect plants growing near the site rather being a signal of food items. Neanderthal sites with plant remains are rare but this is a preservation bias.

Table 2: Neanderthal sites with evidence of macrobotanical plant remains.

Site	Macroremains	Region	Complex	Reference
Douara Cave	Hackberry	Syria	Mousterian	Matsutani, 1987; Griggo, 2004
Gorham's Cave	olive, stone pine	Iberia	Mousterian	Barton et al., 1999
Rabutz	hazelnut	Germany	Mousterian	Toepfer, 1958
Mas-des-Caves	hackberry	France	Mousterian	Barton et al., 1999
Kebara Cave	pistachio, grasses, lentil and other legumes	Israel	Mousterian	Lev et al., 2005
Theopetra Cave	lentil, chickpea and other legumes, grasses and nuts	Greece	Mousterian	Mangafa, 1998

The few existing Middle Palaeolithic sites with botanical remains provide a varied but are incomplete for all inhabited environments (Table 2). At Douara Cave in Syria, abundant deposits of hackberry (*Celtis* sp.) were identified dating to roughly 40 to 55 ka (Matsutani, 1987; Griggo, 2004) Barton and colleagues also reported this plant taxon from Mas-des-Caves in France (1999). Archaeobotanists have found macroremains from stone pine nut (*Pinus pinea*) and olive (*Oliva* spp.) dating to 51,700+3300 BP ^{14}C in the western Mediterranean at Gorham's Cave, Gibraltar. Charred hazelnut shells (*Corylus avellana*) have been identified at Rabutz in central Germany during the warm conditions of the MIS 5e interglacial (Toepfer, 1958), although these shells could have entered the archaeological site by natural processes. Lentil, chickpea, pea and vetchling are reported from Middle Palaeolithic deposits at Theopetra Cave (Mangafa, 1998). The most notable and diverse macrobotanical assemblage so far identified was located in Kebara Cave at Mt Carmel (Israel). This assemblage of charred seeds dates to 63-48 ka, and dwarfs in diversity and size all other Middle Palaeolithic as well as many Upper Palaeolithic macrobotanical assemblages so far recovered. The contents suggest a broad foraging strategy for potential staples, mostly legumes (Fabaceae) with some acorns (*Quercus* spp.), pistachio nuts (*Pistacia* spp.), and chenopods (Lev et al., 2005). Pistachio nuts are rich in lipids, proteins, and carbohydrates, and are therefore an excellent candidate high-rank food, although they would have been available only for a brief season (Dreher, 2012). However, the richness of the legume assemblage is unusual, although protein-rich, they are slow to collect and are arguably low-ranked foods and more usually associated with near-sedentary Epipalaeolithic groups of the Near East (Savard et al., 2006). Many plants were highly restricted by season and would have to be harvested from different habitats in windows from spring to autumn. Overall, the plant remains present evidence of plant use across a variety of Neanderthal habitats, and in at least one case, there are traces of particularly broad use of plants. Yet most macrobotanical examples cannot be unambiguously associated with diet. The collated macrobotanical evidence is promising but overall data are too fragmentary to explore the variation of plant use.

2.5.5 Sedimentological traces of microbotanical plant remains and diet

It is possible to collect data about subsistence patterns from particles in archaeological sediments. Phytoliths, also known as plant opal, are glassy bodies comprised of biogenic silica found in the aerial tissue of plants (Piperno, 2006). They often preserve specific morphologies relating to the plant taxon or plant part that produced them. The decay of the plants releases the phytoliths and thus they enter the archaeological record. Phytoliths routinely survive for vast spans of time and can survive for hundreds of millions of years in certain conditions (Carter, 1999). Thus, they readily survive in the sheltered conditions of cave sediments (Albert et al., 1999). However, if stratigraphic levels are mixed and poorly associated with archaeological evidence it may be difficult link causality of phytoliths to hominin activity. Phytoliths can enter archaeological sites from windblown aerosols, colluvium, bird droppings and other animal activity. Phytolith studies may require well-preserved archaeological deposits examined collaboratively with FTIR and micromorphology. If properly performed, phytolith analyses can provide detailed information about the use of specific plants for many different uses. So far, it has been possible to infer food, bedding, or fuel using phytolith assemblages. Phytolith specialists have studied only a handful of Neanderthal sites for phytoliths. At 58-37 ka cal BP levels in Esquilleu Cave in Cantabria (Iberia), phytoliths indicate continuous deposition of grass leaves by a hearth, suggesting the presence of a bedding zone at this spot (Cabanes et al., 2010). Phytoliths at Amud cave in Israel also indicate plant bedding dating to 70-55 ka (Madella et al., 2002). In some cases, such as Kebara Cave, analysts have retrieved phytoliths from hearth deposits and inferred fuel choice (Albert et al., 2000). Even more interestingly, this site also contained high concentrations of the dendritic morphotype phytolith, which would usually be more familiar in agricultural contexts due to the abundance of domesticated grain. The authors interpreted this accumulation as evidence that Neanderthals repeatedly collected mature grass seed. This is a controversial interpretation because the same pattern could be the result of fauna burying caches of seed. If the anthropogenic interpretation is accepted, it may suggest that Neanderthals in one Levantine site made heavy use of a low-ranked food.

New studies have explored the potential for detecting other anthropogenic indicators in archaeological sediments. Researchers have highlighted that the products of biological processes (biomarkers) may yield insights into the dietary inputs of hominin metabolisms. Most analyses so far have focused on sterols and stanols as candidate faecal biomarkers, because they have the virtue of high stability through the food chains and are resistant to diagenesis (Peters et al., 2005). Only higher mammals form 5β-stanols, which they produce in their intestinal tracts during the metabolic breakdown of cholesterol and phytosterols. Their use as an anthropogenic indicator relies on identifying the source coprolite as hominin. Fortunately for archaeological science, the relative proportions of these stanols and sterols are known to be indicative of dietary preferences although how this works is not understood (Floate, 1970; MacDonald et al., 1983). Sistiaga and colleagues (2014) took sediment samples from morphologically identified coprolites near combustion features in the open-air site of Abric d'El Salt (Alicante, Iberia) dated to 60.7 ± 8.9 and 45.2 ± 3.4 ka (Garralda et al., 2014). These samples were analysed with gas chromatography- multiple reaction monitoring-mass spectrometry. Some samples were dominated by coprostanol and its diagenetic product epicoprostanol, verifying that the samples represented coprolites. The ratio of coprostanol and phytosterol can indicate which taxa produced a coprolite. In all cases at El Salt the values were high, so the authors argued that the faecal residues are from suids or humans, and because no suids were found at the site, humans were the ostensible producers (Sistiaga et al., 2014). The authors report that they discovered from this find that Neanderthals have a high rate of conversion of cholesterol to coprostanol, yet in a fallacy of circularity, use this same trait to identify the coprolite as Neanderthal (Sistiaga et al., 2014). The faecal biomarkers almost certainly represent many separate events and thus cannot be identified to hominin without further biomarkers (i.e. bile acids) (Bull et al., 2002). Yet, although results from El Salt do not reveal diet, multi-pronged faecal biomarkers approaches will likely be highly useful, especially if coprolites deposits are discrete and can be unambiguously identified as Neanderthal.

2.5.6 Evidence of palaeodiet from pathologies

Health is deeply interrelated with diet and many disorders are a result of food choice. In some cases, details on health can be gleaned from surviving hominin skeletal material. Carious lesions are cavities that form on the surface of tooth enamel from the demineralising effects of oral microbiota (Selwitz et al., 2007). The intake of carbohydrates is one of several factors that are needed to form carious lesions (Selwitz et al., 2007) and thus carious lesions can be indirect evidence of diet in contemporary humans. The frequency of these lesions increases relative to the amount of carbohydrates consumed, leading many archaeologists to use this as a proxy for palaeodiet (e.g. Christophersen and Pedersen, 1939; Larsen et al., 1991; Flensborg, 2011). Compared with typical contemporary people, caries are rare in recent foragers (<10 %), but common in past agriculturalist groups (Lanfranco and Eggers, 2012). Dental caries are very rare in surviving Neanderthal teeth (Tillier et al., 1995; Walker et al., 2011b). Of the approximately 1250 Neanderthal teeth examined, just six (0.48 %) have been reported to display carious lesions. Of the six cases, three occur in Iberia, two in France and one in Israel (Lanfranco and Eggers, 2012). Yet seldom do researchers identify caries in early modern humans, although their frequency is unclear. Nevertheless, the rarity of Neanderthals suffering from caries appears to reflect a reliance on animal foods. Not all agree, and the dearth of caries in Neanderthal teeth is taken by some to reflect absence of cariogenic bacterial species in Neanderthal oral flora (Sołtysiak, 2012). For example, molecular evidence suggests that cariogenic bacterial species *Streptococcus mutans* was not present in archaic humans. Yet microscopic examination of dental calculus identified these bacteria from the Kebara 2 and the Subalyuk 1 Neanderthals. In addition, caries may arise from the activities of other cariogenic bacteria species (Tomczyk, 2012).

Another example of a pathology demonstrating diet is dental calculus. The formation of dental calculus is a multicausal process (See 2.6.1), but some dietary factors are clearly involved. Protein consumption enhances calculus formation by increasing the pH of the mouth (Lieverse, 1999). Little effort has been made to extrapolate diet from the abundance of Neanderthal calculus deposits. This is unsurprising as interpreting diet from calculus abundance is challenging. Some agricultural populations with low protein intake have high calculus abundance while some forager populations with ample meat use have little calculus (Lieverse, 1999). This pattern has led some palaeopathologists to use high calculus abundance to trace a high use of carbohydrates instead of protein (Greene et al., 2005). Saliva flow, silicon intake, smoking and predisposition acerbate dental calculus formation in addition to diet explaining this contradiction (Bergström, 1999; Lieverse, 1999).

2.5.7 Evidence of diet from dental wear

Over the course of life, wear reduces the surfaces of teeth. Wear is heavily influenced by the mechanical properties of the food consumed and thus may reveal information on the characteristics of diet (Ungar, 1998). Food may have varied physical properties, such as abrasiveness, toughness, hardness, and brittleness, meaning different foods requires different masticatory processing (Cromton and Hiiemae, 1970; Fiorenza et al., 2011). Over time, attrition, abrasion, and erosion combine to gradually remove the enamel surface of teeth (Kaifu et al., 2003; Addy and Shellis, 2006; Kaidonis, 2008). Attrition is the mechanical force exerted from contact of opposing teeth. Abrasion is another physical wear caused by the rubbing of exogenous material pushed against teeth during mastication. Particles in food such as phytoliths that are softer than enamel are thought to still wear enamel because they force apart the proteins that hold enamel crystallites together (Xia et al., 2015). In the context of Pleistocene foragers, this exogenous matter is predominantly hard and fibrous foods, foreign particles transported in food, and environmental dust carried in wind. Erosion is the chemical dissolution of the tooth surface, but its incidence in the teeth of foragers is insignificant, whereas abrasion and attrition are commonplace (Fiorenza, 2015). Thanks to the gradual advances in our knowledge of the mechanisms of how dental surfaces reform over life, dental wear has matured into a widely applicable means of dietary reconstruction. As the discipline has grown, dental wear has been used to broadly classify the diet of recent forager groups (e.g. El Zaatari, 2010) and the feeding niches of ancient hominins (e.g. Ungar and Sponheimer, 2011). The discipline comprises of two main fields based on the nature of the wear studied - microwear and macrowear.

Dental microwear analysis is the study of the microscopic damage on a tooth's surface as the result of its use. The surfaces of many Neanderthal teeth are highly worn, and this necessitated that early microwear research used the striation patterns on cheek side the buccal surface as a proxy for food masticated on the chewing surface (occlusal surface) (Pérez-Pérez, 1994; Lalueza et al., 1996; Puech, 1999). The buccal wear pattern in individuals consuming a high proportion of meat is characterised by a lower number of striations and a relatively high proportion of vertical ones, while individuals relying on a more vegetarian diet display an increased number of striations, with a greater proportion of horizontal ones (Fox and Pérez-Pérez 1993; Pérez-Pérez 1994). Using scanning electron microscopy, wear specialists (Lalueza et al., 1996) compared Neanderthals with archaic *Homo* specimens and with samples of recent people. Their samples of recent people represented strict vegetarians, tropical and subtropical foragers who consumed relatively high proportions of plant foods, and high-latitude foragers and horticulturalists who consume large amounts of meat. The eight sampled Neanderthals (La Quina V, Gibraltar 2, Tabun 1 and 2, Amud 1, Malarnaud, Saint Césaire and Les Pradelles) had different microwear patterns from those of vegetarians. Some Neanderthals were similar to living groups with high-meat diets, but most fell within the range of both high-meat and more mixed diets. Furthermore, the results taken as a whole showed no compelling chronological, climatic, or geographic patterns.

Buccal and occlusal wear both reflect diet but emerge in different patterns. The formation of occlusal wear is better-understood than buccal wear, making it a more informative approach for ancient hominins (El Zaatari, 2007). More recently, wear studies have grown in sophistication with the integration of confocal microscopy with the advent of "occlusal microwear texture analysis". El Zaatari (2010) used this approach to analyse the occlusal microwear of recent foragers from known temperate, arctic, and other biomes. The groups had varying amounts of marine foods, large game, small game, and plant foods. These samples provided the baseline to which to compare 35 Neanderthal individuals from 23 European and Levantine sites. By grouping these sites by habitat type, El Zaatari showed that Neanderthals associated with mixed and wooded environments consumed plant foods, but in a lesser quantity than meat. Four Neanderthals deriving from open habitats (Spy, La Quina, Arcy-sur-Cure, and Subalyuk) most closely resembled recent groups who fed on fish, seals and guanaco (*Lama guanicoe*) with few plants (about <15 %) (El Zaatari, 2010; El Zaatari et al., 2011). The eight Neanderthals from mixed habitats (Saint Césaire, Petit-Puymoyen, Rochelot, La Chaise, Vindija, Kebara, and Tabun) more closely clustered with marine, small game and plant consuming foragers. The four Neanderthals from closed habitats (Amud, El Sidrón, Grotta Breuil and Zafarraya) had higher levels of molar occlusal complexity and heterogeneity indicating the highest levels of plant use of all the Neanderthal samples. Despite this, they did not cluster with the forest-dwelling recent foragers, tantalisingly suggesting they may not have been as reliant on plants.

The other main approach to wear-based dietary reconstruction is dental macrowear. Molar macrowear represents the cumulative impact of the mechanical properties of diet during an entire lifetime, unlike dental microwear which covers only a brief period just prior to death (Grine, 1986; Janis, 1990). Early efforts to document dental wear interpreted the generality of the abrasiveness of diet rather than the components of diet (Fiorenza et al., 2015). The effectiveness of macrowear studies for dietary interpretations has greatly grown in recent decades with the improved knowledge of how mastication reforms occlusal contact areas (Douglass and DeVreugd, 1997). These developments led Kullmer and colleagues (2009) to develop 3D virtual models to analyse wear patterns on the facets of teeth. By measuring perimeter, inclination, and orientation, it became possible to create a model of how food was chewed, in a method termed "occlusal fingerprint analysis" (Kay and Hiiemae, 1974; Janis, 1990). Fiorenza and colleagues (2011) analysed the occlusal fingerprints of 19 Neanderthals, which he grouped into a deciduous woodland group (Krapina), a steppe and coniferous forest group (Monsempron, Le Moustier and Vindija) and a Mediterranean evergreen woodland group (Amud, Tabun and Shanidar). Wear patterns of the deciduous woodland and Mediterranean evergreen woodland was suggestive of a mixed diet probably containing a significant amount of plants. This group matched wear observed in several plant-reliant recent human reference populations. Neanderthals from steppe and coniferous forest regions exhibited patterns of cold dwelling groups that consume tough foods such as terrestrial mammal muscle or marine foods, though it was not possible to distinguish between these two (Fiorenza et al., 2011). More recently, occlusal fingerprint analysis has explored wear patterns of Neanderthal molars found on the Italian peninsula, Saccopastore 1 and 2, and Guattari 2 and 3 (Fiorenza, 2015). This study found wear suggestive of the use of animal and plant foods on all specimens, though the Saccopastore specimens were more suggestive of meat eating. Guattari 2 fell closer to the previous Mediterranean reference group and Guattari 3 clustered together with the deciduous woodland reference group. Fiorenza and colleagues (2015) interpreted these results as suggesting plant foods had a degree of importance in the warm interglacial MIS 5, while during warm phase MIS 3 Neanderthals appear to have relied more on animal foods.

Micro and macrowear provide a substantial amount of information of the mechanical characteristics of masticated food. From these data, inferences on the nature of diet can be made. However, interpreting these data presents significant problems relating to comparability of environments and differences in reference populations. For instance, the lumping of steppe with coniferous forest biomes makes comparison difficult (Fiorenza et al., 2011). Furthermore, some dental wear is derived not from diet but foreign mineral particles that come in to contact with the enamel, which can confound wear studies (Lucas et al., 2013). Nonetheless, wear-based approaches provide information about Middle Palaeolithic diets unavailable from other lines of evidence. These lines of evidence suggest that Neanderthals consumed plant foods as part of a diet rich in animal foods, and that there may have been more variability across habitats than isotope studies indicate.

2.5.8 Isotopic approaches to palaeodiet

The maturation of methods of dietary reconstruction borrowed from other fields has made major contributions to understanding Palaeolithic diets. The application of stable isotopic analysis to ancient hominins is one example that has become an important means for reconstructing diets and corroborating other lines of evidence (e.g. Codron et al., 2008). Stable isotopic palaeodietary analyses principally use carbon and nitrogen isotopes from collagen from bone and tooth dentine. Isotopes from other elements such as sulphur also can reveal dietary history (Privat et al., 2007). Stable isotopes are analysed as the relative amount of a heavier isotope to a lighter isotope and expressed in δ notation in parts per mil (Schwarcz and Schoeninger, 1991). Carbon isotopes ($^{13}C/^{12}C$) can provide information about the consumption of plants and marine foods, and the nitrogen isotopes ($^{15}N/^{14}N$) reflect use of plants and animals and trophic level. Stable isotopes can sometimes provide detailed information on how much consumed protein was from terrestrial animals, marine animals, freshwater animals or plant foods. Comparison of values with contemporary fauna serves as a reliable means of quality control. The isotopic signal of hominin bone collagen reflects a variable amount of time but given its turnover rate it reflects years of diets in adults (Hedges et al., 2007).

Studies of Neanderthal diets using stable isotopes have garnered much attention. This analysis requires well-preserved collagen and thus is limited by chronology and the taphonomic conditions present at a site. Values have been published from at least 22 individual Neanderthals from 14 sites (See Table 3), and ambiguous values from two others. These sites date between 120 and 30 ka, and are located in France, the Netherlands, Belgium, Russia, Germany, and Croatia (Richards et al., 2000; Bocherens et al., 2005; Beauval et al., 2006; Krause et al., 2007; Richards and Schmitz, 2008; Hublin et al., 2009; Ecker et al., 2013; Wißing et al., 2015). Though isotope and fauna bone studies conflict on Neanderthal prey choice, both indicate that the protein in Neanderthal diets came predominately from terrestrial animals, likely medium and large herbivores (Dusseldorp, 2010, 2013). This isotopic signature has been interpreted as largely representing a consistent ecological niche at the apex of the Pleistocene terrestrial food chain. Isotope values from fauna bones from the same sites suggest that Neanderthals were higher on the food chain than even carnivores such as wolves and bears, but in some cases the comparative fauna was from different archaeological levels and thus may not provide a reliable baseline (Richards et al., 2000, 2008; Bocherens et al., 2005). Some researchers have gone as far to say that isotopically, Neanderthals mimic obligate carnivores (Churchill, 2014). Notably the Saint-Césaire Neanderthal associated with a Châtelperronian tool kit does not differ from the other Neanderthals despite the other indications from this technology that suggest more reliance on plant foods (See 2.5.1). Furthermore, the isotopic values give no indication that Neanderthals consumed aquatic resources like fish or shellfish. The sampled individuals were mostly from inland regions where marine foods are not expected, but the absence of freshwater fish is surprising. Similarly, the Neanderthal isotopic signature appears to leave little room for consumption of plant proteins. However, it cannot entirely rule out a regular intake of protein from plants, due to differences in absorbable protein compared with meat. Plant nutrients such as protein and lipids are often less absorbed than animal equivalents (Baer et al., 2012). Plant foods are typically high in carbohydrates and often contain only moderate levels of protein.

Table 3: Neanderthal remains with published stable isotopic values ($\delta^{13}C$ and $\delta^{15}N$).

Site	N	Age	Region	Predominant diet	Source
Payre [a]	1	MIS 8-7	France	Terrestrial animal	Ecker et al., 2013
Scladina	2	MIS 5c-b	Belgium	Terrestrial animal	Bocherens et al., 1999, 2005
Les Pradelles [b]	5	MIS 4	France	Terrestrial animal	Beauval et al., 2006; Bocherens et al., 2005
Okladnikov	1	MIS 3	Russia	Terrestrial animal	Krause et al., 2007b
Vindija	2	MIS 3	Croatia	Terrestrial animal	Richards et al., 2000
Feldhofer	2	MIS 3	Germany	Terrestrial animal	Richards and Schmitz, 2008
Les Rochers-de-Villeneuve	1	MIS 3	France	Terrestrial animal	Beauval et al., 2006
Saint-Césaire	1	MIS 3	France	Terrestrial animal	Bocherens et al., 2005
Jonaz	1	MIS 3	France	Terrestrial animal	Richards et al., 2008c
Goyet	12 [c]	MIS 3	Belgium	Terrestrial animal	Wißing et al., 2015
Spy	1	MIS 3	Belgium	Terrestrial animal	Bocherens et al., 2001
Engis	1	MIS 3	Belgium	Terrestrial animal	Bocherens et al., 2001
Zeeland Ridge	1	MIS 3	Netherlands	Terrestrial animal	Hublin et al., 2009

[a] This sample derives from tooth enamel and thus has only carbon isotopic values.

[b] The reliability of the isotopic values has been questioned by Bocherens et al., 2005

[c] The twelve samples represents four or more individuals.

Neanderthals may have plausibly targeted vegetal foods low in protein and high in carbohydrates and lipids to ameliorate the risk of protein poisoning (See 2.4.1). We cannot quantitatively estimate contributions of each component of diet unless a mathematical model (mixing model) is used (Bocherens, 2009; Fernandes et al., 2014). Reliably fitting of such models requires the input of isotopic values of all the consumed foods, and this is not possible in a Palaeolithic context. Therefore, mixing models in these contexts might be misleading.

Some have challenged the view that Neanderthal protein intake was near exclusively animal-based. Specialists have explored various possibilities to assess if the nitrogen isotopic values reflect an extremely high trophic level. Speth (2010) noted that severe nutritional stress can lead to increases in $\delta^{15}N$ due to the effects of protein catabolism, and that this starvation signature may explain the elevated Neanderthal $\delta^{15}N$ signal. Bouts of starvation are a well-documented part of life for some foragers, particularly those in high latitudes such as Arctic foragers. Episodes of stress (nutritional or illness related) endured by Arctic foragers in childhood are visible with enamel defects such enamel hypoplasia on their teeth. Comparison of Arctic foragers and Neanderthal indicate comparable stress levels (Guatelli-Steinberg et al., 2004). However, given the slow turnover rates of bone, malnutrition severe enough to elevate δ^{15}N is would normally be fatal before it could be recorded in bone (Beaumont et al., 2013), suggesting that malnutrition might not explain the Neanderthal signal.

Another source of confusion may come from the fauna used as a baseline for extrapolating Neanderthal trophic level. If prehistoric faunal diets differed from that of their present day counterparts, it may confuse our interpretation of Neanderthal diets. For example, if the herbivores that Neanderthals consumed had elevated δ^{15}N, then the Neanderthal trophic level would appear high. This would mask consumption of plant protein if comparative fauna were unavailable. Usually ideal numbers of comparative fauna from the same levels are absent from isotopic studies. Furthermore Eurasian elephantids have unusually high δ^{15}N values unlike present day elephants (likely relating to consumption of faeces), and some have suggested that this explains the apparent trophic level of Neanderthals (Richards et al., 2000; Bocherens et al., 2005; Kuitems et al., 2012). However, elephantid consumption is unlikely to explain high δ^{15}N values for all individuals published. Neanderthals consumption of elephantids appears to have been rather limited (See 2.5.3), while the Neanderthal isotope values are remarkably consistent across samples. The consumption of high δ^{15}N nitrogen prey may have had an impact, but the potential to distort Neanderthal isotopic signals should not be overstated in this case. Although isotopic studies have given a powerful insight into protein consumption, these sampled Neanderthals are disproportionately from northern open environments and cold phases (Richards and Trinkaus, 2009; Salazar-García, 2012). Few southern Neanderthal isotopic values have been published, in part due to the poorer preservation of Neanderthal collagen in these warmer climates (e.g. Ambrose, 1990). Of the published 22 Neanderthals subject to collagen isotopic studies, only two individuals lived in the forested interglacial period (MIS 5), while others are derived from a range of environments from climatic phases. This biased sample should temper interpretations based on isotopic data and reveal little about dietary variation in different environments.

2.5.9 The contribution of dental calculus to understanding Middle Palaeolithic diets

One the most exciting emerging ways to learn about ancient diets is to use hominin dental calculus. Dental calculus along with dental enamel is the only tissue in the human body with no means of regulated shedding. This unique characteristic enables entrapment and preservation of food particles and other materials become entrapped in this biomineral deposit following consumption. Such an approach could address how Neanderthals used plants. Dental calculus (tartar) is dental plaque that has become calcified by salivary calcium phosphate (Lieverse, 1999). It is a ubiquitous pathology of the mouth in humans and human relatives. Researchers have reported finding starch grains, phytoliths, pollen, diatoms and other particles relevant to life history entrapped in human dental calculus for extended periods of time (e.g. Dobney and Brothwell, 1988; Boyadjian, 2012). Dental calculus sampled from living or dead individuals is rapidly gaining recognition as an invaluable material for the reconstruction of life histories. The integration of dental calculus analyses to Palaeolithic hominin remains has made powerful contributions to our knowledge of Neanderthal diets. As dental calculus offers direct evidence of the plants that entered the mouth, this analysis can potentially give information of plant use that is invisible with other methods.

Henry and colleagues (2011) pioneered the application of this approach to the elucidation of Neanderthal diets. This study used dental calculus sampled from one individual from Shanidar Cave, Iraq, and from two Neanderthal individuals from Spy Cave, Belgium. These analyses recovered remains of phytoliths from date palms (*Phoenix* spp.) and starches from grass seeds (Triticeae), legumes (Faboideae) and potential indeterminate underground storage organs. Although the assemblages probably reflect consumed foods it is difficult to rule out the contribution of chyme (semi-digested stomach contents). Chyme is widely consumed by foraging societies and it probably was a feature of Middle Palaeolithic diets (Buck and Stringer, 2014). However, starches predominated in these samples, yet ungulate chyme would predominately contain phytolith not starches, arguing against chyme being the primary source of plant remains in dental calculus.

Some of the starch grains, including Triticeae starches, were apparently partially disrupted (semi gelatinised). Gelatinisation is a process where starch undergoes a breakdown of its intermolecular bonds when heated in the presence of water. Thus, semi gelatinisation was interpreted as evidence of the controlled boiling and cooking of these starches. Yet some have queried whether this process could occur spontaneously to starches trapped in calculus for tens of thousands of years (Collins and Copeland, 2011). Current research is investigating the tempo of spontaneous gelatinisation and it remains to be seen if we can ascertain if Neanderthals boiled Triticeae seeds.

Another study sampled dental calculus Neanderthals from El Sidrón in northern Iberia, where a rich assemblage of Neanderthal remains was found. Hardy and colleagues (2012) analysed dental calculus from five Neanderthals dating to 51,100 ka. Notably, this sample is probably the only example from temperate woodland environment so far published. Hardy recovered moderate numbers of starches in four of the five samples and one grass phytolith in one of them using optical microscopy. In addition, the dental calculus samples were analysed with (thermal desorption and pyrolysis) gas chromatography-mass spectrometry to assess if compounds relating to diet could be present. This technique yielded molecular evidence of inhalation of wood smoke and naturally occurring bitumen. The presence of compounds such as chamazulene, dihydroazulene, and 4-methylherniarin suggested exposure to yarrow and chamomile herbs. Hardy interpreted this as traces of plants used for medicinal rather than for nutrition purposes. Critics have raised concerns that these herbs may enter diet in chyme (Buck and Stringer, 2014). Pleistocene ungulates probably commonly grazed on yarrow. In addition to undeliberate use in chyme, plants like yarrow and chamomile may have been gathered and consumed as a vegetable. Although bitter-tasting, Buck and Stringer (2014) point out that traditional Alaskan people consumed camomile as a food plant (Kuhnlein and Turner, 1991). All food types may enter the mouth in many alternative ways such as ritual uses, dental hygiene or accidental intake.

Later published research has attempted to address the lack of data on Neanderthals from Mediterranean environments. Within a multi-proxy research article about Neanderthal diets from eastern Iberia, the author analysed dental calculus from nine teeth and tools from Sima de las Palomas del Cabezo Gordo in southeast Iberia (Salazar-García et al., 2013). To control for contamination, wash samples of cave rock fall (éboulis) were collected, and these showed grass phytolith and some starch contamination traces. However, these control stones were selected from a unit balk exposed to atmospheric airborne microremains for an extended span of time. Calculus and sediment adhering to fauna teeth from the site were also examined, analysis found no starches and only three phytoliths, mostly coming from grasses. Unlike the controls Neanderthal calculus recovered microremains types included leafy matter indicated by polyhedral multi-cells, hard endosperm of seeds or nuts as well as grass seeds and possibly underground storage organs. The starch grains found in dental calculus samples largely overlapped with the types recovered on the stone tools, although this overlap may be overestimated due to the lack of universal starch classification types in the discipline.

These studies have provided some information on elements of Neanderthal foraging strategy and show that Neanderthals were capable of sourcing nutrients from a variety of plant foods. However, these findings only constitute qualitative information from archaeological sites dispersed by space and time. More recent work by Henry and colleagues (2014) attempted to explore Neanderthal plant use by comparison to African Middle Stone Age and Near Eastern and Europe Upper Palaeolithic peoples. This research used both dental calculus samples and wash samples of the surfaces of stone tools from Neanderthals, African Middle and Later Stone Age, and Eurasian Upper Palaeolithic peoples. A Poisson mixed model was used to test if Middle Stone Age and Middle Palaeolithic groups used less plants than Upper Palaeolithic and Later Stone Age peoples, using number of plant types as a metric of diet breadth, and controlling for the effects of geographic region, stone tool type and sites. This model suggested that all of the considered groups consumed an approximately comparable array of plant foods and none of the expected parameters of variation (stone tool industry or geographic region) had a significant influence on the number of plant species consumed. There was also no apparent pattern in plant use through time. Fundamentally, the results failed to detect any difference between Neanderthals and any modern human group.

These studies have indicated the potential for dental calculus research to reveal foods, in particular the use of low-ranked underrepresented food sources (e.g. Triticeae) and details about the breadth of plants consumed. However, there are many aspects of the calculus record that must be considered when applying this method to Neanderthal samples. In the following two sections, I discuss issues faced when interpreting the dietary signal of dental calculus.

2.6 The state of the art in dental calculus research

2.6.1 A background

The study dental calculus has a long history in archaeological research. Archaeological dental calculus has been noted as a pathology in studies of health of past populations since early decades of the 20th century (Leigh, 1925; Hughes, 1963). It was long recognised that this pathology is intertwined with diet, and the incidence of dental calculus was studied as a proxy for the amount of carbohydrates or proteins ingested (See section 2.5.6). The potential of dental calculus to open a door to specific dietary choices of past populations was first noticed in the 1970-1980s. Dobney and Brothwell (1986; 1988) demonstrated that dental calculus could yield data on the diets of human populations. Today, analysis of plant and animal microremains recovered from archaeological dental calculus has grown to become a widespread means of aiding dietary and health reconstruction (Boyadjian et al., 2007; Blondiaux and Charlier, 2008; Henry et al., 2011; Liu, 2012; Mickleburgh and Pagán-Jiménez, 2012; Warinner et al., 2014; Power et al., 2016). Microscopic plant remains preserved in dental calculus can inform us about the exploitation of plants otherwise invisible to us, thereby enabling us to obtain direct information on a wide variety of question on prehistoric societies. For example, plant microremains from dental calculus have indicated the use of beans in a complex plant diet in South America (Piperno and Dillehay, 2008), described early agricultural diets at Tell al-Raqā'i, Syria (Henry and Piperno, 2008), and recorded pre-Columbian Caribbean subsistence (Mickleburgh and Pagán-Jiménez, 2012). Dental calculus has also contributed to dietary studies of the early African hominin *Australopithecus sediba*. Phytoliths found in the dental calculus of the MH2 individual suggested a C_3 diet incorporating dicotyledons (tree leaves, fruits, wood and bark) and monocotyledons (grasses and sedges) (Henry et al., 2012). More recently, dental calculus has been used to examine characteristics of diet from Lower Palaeolithic hominins at Qesem Cave in Israel (420–200 ka). Hardy and colleagues (2015) used starch grains and specific chemical compounds recovered from dental calculus to infer the ingestion of plant foods. They interpreted pollen, fungal spores, microcharcoal and invertebrate remains as evidence of the inhalation of respiratory irritants.

2.6.2 Technical difficulties in current approaches in dental calculus research

Despite this growing interest in dental calculus as a source of ancient dietary information, dental calculus is still poorly understood. Dentistry research has paid scant attention to the mechanisms by which plant microremains become trapped and preserved within calculus. Native starch grains (i.e. starches in their original unaltered state) are the predominant focus of much of the microbotanical archaeology literature (Mickleburgh and Pagán-Jiménez, 2012; Leonard et al., 2015; Tao et al., 2015). Yet there has been a lag in explaining how starch grains and grain morphology persist in archaeological contexts (Haslam, 2004; Torrence and Barton, 2006; Hardy et al., 2009). Starch is a biodegradable molecule and it should rapidly degrade after burial (Hardy et al., 2009; Langejans, 2010; Henry, 2014). Starch does seem to survive in certain situations, as unambiguous ancient starch is found in archaeological contexts (Samuel, 1996). The survival of ancient starch presumably reflects protective qualities of its semi-crystalline polysaccharide structure and specific microenvironment conditions that isolate starches from taphonomic processes (Hardy et al., 2009; Salazar-García et al., 2013; Henry et al., 2014). However, phytoliths are far more robust than are starches and routinely survive in most sediment types, yet they may dissolve when exposed to a high pH. It is poorly understood how the alkaline environment (pH ≤ 9) of dental calculus affects phytoliths (Kleinberg, 1970). Other botanical microremains that are useful for archaeobotanists have similar problems. Calcium oxalate crystals (calcium phytoliths) are a category of microremains found in energy-rich plants. Problematically, calcium oxalate crystals are susceptible to dissolution even in mild acids. The acidity of saliva may readily drop low enough to dissolve calcium oxalate present in the mouth (Tromp, 2012). The other types of microremains that may be present in dental calculus such as pollen, may well be subject to comparable problems.

Dental calculus could offer these microremains a secure mineralised matrix where they become embedded and entirely isolated from soil chemistry and microbial action (Warinner et al., 2014). Of course, to even reach this point, starches that have entered the mouth must first survive breakdown in the oral cavity. The mouth is a hostile environment for exposed starch grains because of the action of salivary digestive enzymes and bacterial metabolic activity that will rapidly attack and digest starches (Lukacs and Largaespada, 2006). Most digestion of starch occurs at a later point in the digestive system due to the effects of pancreatic amylase, but the high amounts of salivary amylase found in most human groups may still have an impact. We may only speculate that some starch avoids oral enzymatic digestion and is stochastically forced into protected niche areas of calculus. Alternatively, it could be explained in a slower model, where starch (resistant starch, higher in amylose content than typical starches) evades digestion and is gradually precipitated into dental calculus (Hardy et al., 2009).

There has been little attempt to examine mechanisms that may be involved. Regrettably, the conventional methodologies in dental calculus analysis rely on invasive sampling of calculus from the tooth, making this harder to study. They involve mechanically or chemically removing dental calculus from the enamel surface, grinding or dissolving it to break up the sample, and finally examining the particles using optical light microscopy (Henry and Piperno, 2008). Due to this extraction, microremains observed by the analyst are no longer in context in calculus. This is unfortunate, because the microenvironments that seem to preserve microremains in dental calculus may shed light on whether microremains are not lab contamination, the mechanisms of microremain preservation, and if they are damaged in extraction. Due to this, Weyrich and colleagues have questioned reproducibility and accuracy of dental calculus studies using microremains, pointed to sedimentary contamination as undermining these studies (Weyrich et al., 2015).

2.6.3 The representativeness of the dental calculus dietary record

Perhaps the most serious problem in dental calculus dietary studies is the ambiguity of what can be inferred about diet from the plant microremain record found in dental calculus. Researchers can say surprisingly little about how representative this record is, despite the plethora of studies using dental calculus as a source of dietary information. Little research has attempted to quantitatively cross-validate the dietary material recovered in dental calculus with the organism's actual feeding ecology and life history. Detailed studies using controlled diets have not been pursued and there are few published studies using faunal dental calculus where diet may be reliably predicted (Armitage, 1975) . Many past studies have assumed that the plant matter preserved in dental calculus representatively reflects a long-term dietary average (Mickleburgh and Pagán-Jiménez, 2012). Yet we should question this assertion for many good reasons. Plant remains trapped in dental calculus could plausibly be derived from airborne particles, water, chewing of plant matter for health or fibre processing, amongst other possibilities. This raises the prospect that microremains found in dental calculus from the studied Neanderthal samples may not in fact reflect plant consumption at all.

Knowledge of the timing of the formation of the calculus dietary signal would greatly assist life-history based approaches. Dental calculus does not form and accumulate in a continuous and predicable way (Lieverse, 1999). Soft dental plaque can make the transformation into hard mineralised calculus over the course of weeks, but mineralization may be episodic and the rate it occurs varies among individuals according to age, oral hygiene, nutrient intake (Bergström, 1999; Lieverse, 1999; Jin and Yip, 2002) and possibly also genetic predisposition among other things. In addition to these complications, dental calculus deposits can become dislodged from the enamel at any point during life, resetting the dental calculus dietary record along with it.

Leonard and colleagues examined dietary representativeness using living Namibian Twe forager-horticulturists (2015). The Twe retain a partially traditional diet in a mountainous arid habitat. Twe grow maize (*Zea mays*), pearl millet (*Pennisetum glaucum*), squash (*Cucurbita* sp.), melons (Cucurbitaceae spp.) and sugarcane (*Saccharum* sp.). Although they still collect a variety of wild plant foods, since 2006 an increasing proportion of their diet is from government-supplied maize meal. Leonard and colleagues established dietary patterns through interviewing and observing food choice during in short-term camp stays. Leonard and colleagues noted that older individuals and males had larger dental calculus deposits than young people (under 35 years old) and females, a potentially confounding affect. The number of microremains per individual poorly predicted the range of starch and phytolith producing plants consumed. Nineteen Twe vegetal foods contained starch and phytoliths, but no calculus sample contained more than six plant food types. A population approach was more successful, but Leonard and colleagues stressed that in her population, a sample of 50 individuals or more is needed to have 95 % confidence of observing several foods. Overall, their analysis suggested that starch grains and phytoliths in Twe dental calculus gave an incomplete picture of diet and significantly underrepresented vegetal foods (Leonard et al., 2015). It is unclear if these results can be applied to prehistoric hominin populations. It is unknown how many copies of *AMY1* the Twe people have so we cannot assess if this influenced the results. Despite this study's valuable contribution, this study lacked insight into the long-term dietary history of the studied individuals.

2.6.4 Outlining the findings for dental calculus research and Neanderthal diet

Sections 2.1 through 2.3.2 provide details why reconstructing diet is an essential part of studying both human origins and the life history of Neanderthals. A behavioural ecology framework is a very powerful means for achieving this task. By using diverse array of approaches, I have demonstrated what can be extrapolated from current evidence about the Neanderthal diets. I have assessed the crucial issues that must be resolved to move forward to a more complete palaeobiology of these hominins. This dissertation is based on using plant consumption to test how adaptable Neanderthal diets were. Dental calculus analysis was selected to provide a window on the plant use of Neanderthals. The unanswered questions highlighted by this introductory section form the basis of this PhD, which includes three main parts: are part of this PhD; a revision of how we obtain dietary data from dental calculus, a re-evaluation of the resolution of the dental calculus dietary record and a new measure of plant foods and dietary breadth for Neanderthal diets.

The first paper highlights the problems of conventional dental calculus research. It examines dental calculus from wild chimpanzees and archaeological specimens first with scanning electron microscopy–energy-dispersive X-ray spectroscopy, and then with conventional optical light microscopy to compare techniques. This allowed for the first time investigation of the microenvironment that traps starch and other microremains. Lastly, it developed a sequential workflow that maximises the amount of life history information extractable from dental calculus. The second paper focuses on associating debris of plants inside dental calculus to diet and behaviour. It aims to address the troubling gap between plants in dental calculus and dietary records by using calculus of chimpanzees with documented diets. Samples of chimpanzee dental calculus (Taï National Park, Côte d'Ivoire) showed that microremains accumulate as long-lived dietary markers. The paper found that phytoliths allow feeding preferences of the chimpanzees to be reconstructed, while starches do not. Microremains also implied that assemblages could record population information about other dietary behaviours, such as the age of weaning and learned food processing techniques like nut cracking. Finally, the third paper uses dental calculus from Neanderthal remains to provide new light on plant exploitation from a mix of environments. Dental calculus was analysed from five archaeological sites: Vindija (Croatia), Grotta Guattari (Italy), Grotta Fossellone (Italy), Sima de las Palomas del Cabezo Gordo (Spain) and Kalamakia (Greece). These sites represent a variety of regions and biomes across Europe. Starch, phytoliths and other microremains suggested Neanderthals used a wide variety of plants including low ranked plant foods. The findings were then combined with data from past studies to model if local vegetation, winter temperature or the age of the site account for variation in diet. The model found local vegetation and winter temperatures do not influence the patterns in the dental calculus data suggesting that although Neanderthal consumed they have had an inflexible but partially broad dietary adaptation.