Geochemical analysis of Cenozoic fossil conifers at high latitudes: Implications for molecular preservation and environmental change

The Honors Program
Senior Capstone Project
Student's Name: Caitlyn Witkowski
Faculty Sponsor: Hong Yang
April 2010

Table of Contents

ABSTRACT

Fossil materials record ancient life and their adapted environment. Arctic plant fossils are critical for our understanding of the Earth's paleoenvironment when high latitudes were under ice-free conditions. All Arctic plant fossils in this research are conifers, plants conducive for morphological and molecular study because of their incredible genetic stability. Miocene (15 million year old) and Pliocene (5 million year old) conifer leaves were collected from Banks Island, Canada (Williams et al., 2008). Samples were analyzed and compared with Paleocene (60 million year old) and Eocene (45 million year old) samples from Axel Heiberg Island, Canada and with modern equivalent species from Washington D.C., USA (William et al., 2008). This paper has three main sample analyses. First, Pyrolysis-Mass Spectrometry-Gas Chromatography technology was used to detect organic volatile compounds. The amounts and types of organic volatile compounds provide further insights into the molecular preservation of the Miocene and Pliocene fossilized samples. Molecular preservation from this research was compared to previous research that used Scanning Electron Microscope observations of Paleocene and Eocene transverse sections to indicate extraordinary morphological preservation (Yang et al., 2005; Yang et al., 2007). Second, Miocene and Pliocene bulk peat were cross-referenced with known species in the region to reconstruct Arctic environmental changes between 5 million and 15 million years ago. Third, the ratios of three stable compounds were analyzed as biomarkers, essentially benchmarks for plant fossil preservation. However, biomarkers were inconclusive because of complications including age, species type, and environmental conditions. Overall, our analyses provide the first assessments of molecular preservation for these rare Arctic fossils which offer unique material for further paleoclimate analysis.

INTRODUCTION

Previous research has questioned whether there is a correlation between morphological preservation and molecular preservation (Eglinton and Logan, 1991; Stankiewicz et al., 1997; Briggs et al., 2000; Yang et al., 2005). In order to better understand the relationship between the two types of fossil preservation, collaborative investigations have provided considerable insight into the means by which biomolecules are responsible for extraordinarily preserved morphological features in fossil samples[1]. In this paper, the molecular preservation of these samples is examined in relation to the morphological preservation. In order to provide a working background, first, the significance of these samples, including the importance of the research to science, the reason for examining these specific genera, and the uniqueness of these particular analytes was examined. Second, morphological preservation and the methodology behind Scanning Electron Microscope (SEM) observations, as well as the significant findings in previous related research are discussed. Finally, molecular preservation and the methodology behind Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS) analysis are examined.

Extraordinarily well-preserved fossil deposits, also known as *lagerstätten*, are essential to providing insights into paleoecological conditions. Understanding paleoecological conditions contributes invaluable information to the understanding of the past. Individual sample analyses add to the collective knowledge of a region, the continent, and ultimately the world. A clear comprehension of Earth's past, from plate tectonics to ocean currents and climate patterns, provides insight into the changes in our modern and potential future environment. Understanding how the Earth functions over time will help humanity prepare for the future, particularly in relocation projects, disaster control, and agricultural planning.

---

[1] Fossils are mineralized remains of ancient biological matter. The material examined in this paper has not completely mineralized; the analytes contain organic compounds, known as exceptionally preserved compression fossils or Fossil *Lagerstätten*. Stating that the samples are simply leaves understates the remarkable age of the samples, so for the purpose of this paper and for the lack of a better term, the 5-45 million year old organic conifer samples will be referred to as fossils.

In addition to reconstructing ancient environments, *lagerstätten* offer a better understanding of the fossilization process via the evidence of morphological characteristics for biochemical analysis of the ancient *in situ* biomolecules in these fossils. Scientists determine whether a sample is *lagerstätten* from its relative preservation compared to other samples. Although there are criteria to determine a sample's preservation, such as the presence and abundance of polysaccharides, there is no specific number to quantify a sample as *lagerstätten*. Morphological and molecular analyses are qualitative measurements. Samples examined in this research have been determined to be *lagerstätten* based on their remarkable preservation in a comparative analysis to fossil samples from different time periods and from different sites.

These specific samples are also significant. All of the samples examined in this research are conifers. Conifers are cone-bearing trees and shrubs typically found at high latitudes in the Northern Hemisphere. Because of their incredible genetic stability, conifers are often examined as paleoecological indicators via their morphological and molecular preservation. Scientifically known as the Cupressaceae genera (such as *Glyptostrobus*, *Taxodium*, and *Metasequoia*) and as the Pinaceae genera (such as *Larix* and *Pinus*), conifers have remained exceptionally static, both structurally and molecularly, since the Cretaceous period between 145.5 – 65.5 million years ago (Chaney, 1951; Liu et al., 1999). In addition, Cupressaceae and Pinaceae provide long and well-documented fossil records; the comparative availability of both fossil and modern conifers increases its accessibility for research.

The remarkable preservation of these conifer fossils is the result of the particular conditions in which the samples were buried. The Arctic has remained relatively untouched by the forces of nature, flora, fauna, and man because of its cold, dry environment. As a result, there is an abundance of conifer samples from the Cenozoic. Ancient conifers are compared to modern conifers to reconstruct the environment and better understand the progression of the Earth's history. Conifer fossil record in Cenozoic deposits of the Arctic region provided unique material for paleoclimate studies. For site location, please see Appendix A. For chronological geological time period table, please see Appendix B.

MATERIALS

Both modern and fossilized conifer leaves are shown alphabetically by genus name in Table 1, including genus, sample abbreviation, locality, and approximate age. Mid-Eocene *Glyptostrobus* and *Larix* samples were collected from the Upper Coal Member of the Buchanan Lake Formation, Axel Heiberg Island, Canada located at 79°54'55.8"N, 89°01'26.8"W (Ricketts and Stephenson, 1994; Yang et al, 2005). Mid-Miocene and Pliocene samples, including bulk peat, *Glyptostrobus*, *Larix*, *Metasequoia*, *Picea*, *Pinus*, and *Taxodium*, were collected from Ballast Brook Formation, Banks Island, Northwest Territories, Canada located at 74°18′N, 123°02′W (Williams, 2008). *Metasequoia* and *Taxodium* were collected from the Clarkia Miocene deposit in northern Idaho, UAS (Yang et al., 2005). United States National Arboretum in Washington D.C., USA located at 38° 54′ 43.56″ N, 76° 57′ 49.68″ W provided the modern samples (Williams et al., 2008).

Genus	Sample	Locality	Time Period	Age in Years
Glyptostrobus	66	Washington DC, USA	Modern	3
Larix	68	Washington DC, USA	Modern	3
Picea	CW-F	Alaska, USA	Modern	3
Pseudo larix	65	Washington DC, USA	Modern	3
Bulk peat	B7	Banks Island, CAN	Pliocene	5 million
Picea	B6	Banks Island, CAN	Pliocene	5 million
Bulk peat	B5	Banks Island, CAN	Mid-Miocene	15 million
Glyptostrobus	B2	Banks Island, CAN	Mid-Miocene	15 million
Larix	B3	Banks Island, CAN	Mid-Miocene	15 million
Metasequoia	B8	Clarkia, Idaho, USA	Mid-Miocene	15 million
Pinus	B1	Banks Island, CAN	Mid-Miocene	15 million
Taxodium	B9	Clarkia, Idaho, USA	Mid-Miocene	15 million
Glyptostrobus	49	Axel Heiberg Island, CAN	Mid-Eocene	45 million
Larix	51	Axel Heiberg Island, CAN	Mid-Eocene	45 million

Table 1- Conifer leaf samples used for Py-GC-MS

Based on the analysis of samples collected and based on current environmental paleoreconstruction, prevailing Cupressaceae gymnosperms and broad-leaved flora grew under a humid, temperate, or possibly warm-temperate climatic conditions (Yang et al., 2005; Basinger, 1991; McIver and Basinger, 1999; Jahren and Sternberg, 2003). The original inferred paleoenvironment has recently been questioned based upon further projections of forest biomass and productivity. Fossil evidence proposes that early flora in the Canadian Archipelago parallels modern old-growth forests, suggesting that the ancient environment experienced cold-temperate conditions (Yang et al., 2005; Williams et al, 2003). Furthermore, early and modern conifers are similar in physiological ability to endure freezing temperatures, supporting that the paleoenvironment likely included cold, dark winter months as currently experienced at high-latitudes. For map of sample location, please see Appendix B.

Mid-Miocene and Pliocene fossils were collected from the exposed Ballast Brook Formation along the walls of the Ballast Brook Valley on northwestern Banks Island by Williams. The Ballast Brooke Formation has been classified by five different units: Units 1, 2, 3, and 5 are of flat lying sandy and silty-clayey strata and Unit 4 is of 3 meter-thick and 15 kilometer-long autochthonous peat bed rich with fossils. Although the peat bed provides invaluable, several million year old plant matter, it is nearly impossible to date because there is no radioactive material or rock formations to analyze. Currently, all estimates for the sites age are based on relative dating which assumes age based on similar fossils from other age-known sites (Williams et al. 2008).

Unit 4 provided samples of bulk peat (B5), *Glyptostrobus* (B2), *Larix* (B3), *Pinus* (B1), and *Taxodium* (B9). It is subdivided into low-moor peat and high-moor peat. Peat is the accumulation of partially-decayed vegetation and is often found in bogs and swamp forests; autochthonous peat is formed by the gradual accumulation of plant remains in water, likely from a fluctuating water table. Current hypotheses suggest that a fluctuating water table would prevent material from fossilizing while also preventing samples from decomposing, thus explaining how 5-15 million year old conifer leaves have neither fossilized, which can happen within several thousand years, nor decomposed, which can happen within only several months. Ballast Brook Formation sediments accumulated on the valley floor of a meandering

river system and included overbank, backswamp, and floodplain-pond facies, further suggesting the likeliness of a fluctuating water table in this region (Williams et al, 2008).

Below in Figure 2, A displays a long-shot of the formation and overlying the Beaufort Formation outcrop near the type section described by Fyles et al. (1994). The arrow points at Unit 4, the autochthonous peat bed, characterized by the dark band in middle of the outcrop. B provides a close-up of the Ballast Brook autochthonous peat in Unit 4. The material consists of bulk peat, dry and partially-decomposed material from the Pliocene and Miocene. More detailed geological information on Banks Island, Canada may be found in Williams et al (2008).

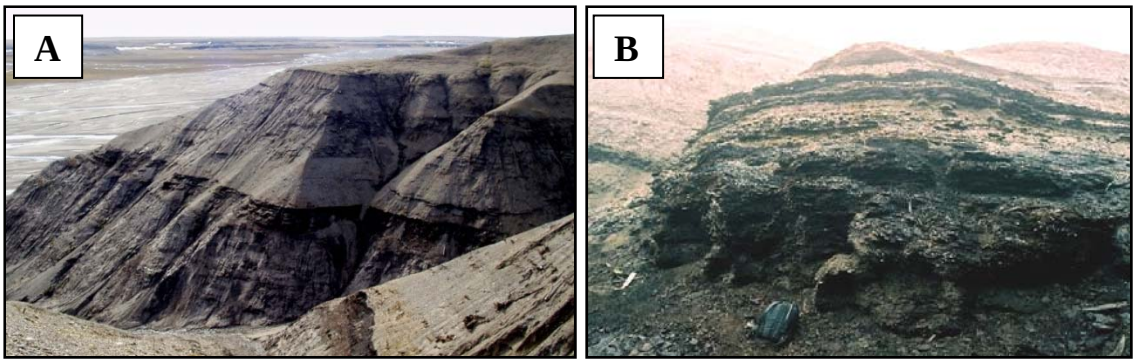


Figure 2 - Ballast Brook Formation, Banks Island, Canada

Metasequoia dominates plant fossil remains in mid-Eocene Axel Heiberg Island and commonly co-exists with *Glyptostrobus* (such as sample 49), another member of the Cupressaceae. Other similar species were also found, including *Larix* (such as sample 51), *Pseudo larix*, *Picea*, *Pinus*, and Tsuga (Basinger, 1991; LePage and Basinger, 1991; LePage, 2001). The Upper Coal Member of the Buchanan Lake Formation is believed to be a fluvial deposit from 41.3-47.5 million years ago when the Uintan North American land mammal stage first began (Yang et al., 2005; McIntyre, 1991; LePage, 2001). More detailed geological information on Axel Heiberg Island, Canadian Arctic Archipelago, Canada may be found in Ricketts (1986) and in Ricketts and McIntyre (1986).

METHODS

Two major types of methodology were used for examining the preservation of samples. First, scanning electron microscope (SEM) was used in previous research to examine the morphological preservation of the samples. Secondly, pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) was used in this research to examine the molecular preservation of the sample. The results of the two methods were then compared.

Morphology and SEM

Every organism in nature has specific characteristics and structures that discriminate it as a particular species. In science, morphology is the study of the biological structures that distinguish species. Different fields within morphology include comparative morphology (the analysis of structure patterns within an organism), functional morphology (the study of the relationship between structure and function), and experimental morphology (the study of the effects of external factors on an organism's morphology). Comparative morphology via scanning electron microscope (SEM) observations was used to determine the species type of the fossilized plant samples and, later in comparison with modern equivalent species, each sample's relative preservation. SEMs, unlike most microscopes, use electrons instead of light to capture images and use magnets instead of lenses to view samples. The machine scans the surface of a sample using a high-energy beam of electrons in a raster scan pattern. Data registers based on the way in which electrons from the high-energy beam interact with the atoms of the sample (Schweitzer, 2010). SEM is more effective than simply observing a sample under a light microscope because it provides data on the surface topography, composition, and electrical conductivity.

Leaves were sliced to provide a traverse (horizontal) section of the sample in order to view the structure of the cell wall. Using SEM methodology to examine the fossilized samples, no gross morphological differences were observed among samples from different Arctic islands (Yang et al., 2005). Based on the amount of degradation to the sample, such as deformities and lack of major structures in the cell wall, sample preservation was ranked in relation to previously found samples (Leng et al., 2010; Yang et al., 2005). The Arctic samples were relatively similar to plant taxa from the Clarkia site; all samples lack major morphological degradation, though Clarkia samples were slightly more compressed than the Arctic samples

(Yang et al., 2005; Smiley et al., 1975; Smiley and Rember, 1985). For selected SEM observations please see Appendix C.

SEM observations determined the morphological preservation of the samples; this research further explores the preservation of these same samples, but now on a molecular scale. In order to determine the molecular preservation, analytes were examined using Py-GC-MS.

Organic Chemistry and Py-GC-MS

Samples were first prepared before running them through pyrolysis-gas-chromatography-mass spectrometry (Py-GC-MS) methodology. All samples were washed with de-ionized water and air-dried at room temperature. De-ionized water is used to prevent the contamination of the sample, particular with organic material and sediments. Samples were then freeze-dried and crushed using a mortar and pestle. The freeze-dry process eliminates excess water from the sample in order to better extract lipids from the sample and to minimize any water contamination from the surrounding atmosphere. Standard ultrasonication was used for fifteen minutes with 2:1 (v/v) dichloromethane (DCM): methanol (MeOH), an organic extraction solvent. ASE 300 (Accelerated Solvent Extraction) technology removed free lipids from the leaf samples. Lipids are removed for easy access to the rest of the plant tissues. When lipids are not removed, Py-GC-MS analyses become extremely difficult; lipid extraction prevents overcrowded chromatographs, increases ease of compound identification, and makes area ratios of each compound feasible. The lipid-free insoluble residues were examined using Py-GC-MS.

Py-GC-MS is the scientific method that combines three instruments for the thermal decomposition (pyrolysis), the separation (gas chromatograph) and the identification (mass spectrometer) of compounds in a given sample, known as an analyte. The tri-equipment method provides both qualitative and quantitative data for sample evaluation (McMaster and McMaster, 1998). The concepts behind the technologies were developed over a century ago; however, applications of the technologies weren't prevalent until the 1940s, the combined use of GC-MS didn't exist until the 1950s, and the full combination of Py-GC-MS wasn't prevalent until the past decade (Gudzinowicz et al., 1977). The sum of the combined Py, GC, and MS is greater than the whole of each individual machine. Because GC-MS has become

less expensive and more readily accessible, precise, and accurate, it has become increasingly popular in many fields of science; modern applications of the method are extensive. GC-MS spans into nearly every chemistry discipline, including medicine, forensics, security, environmental analysis, and astrochemistry (McMaster and McMaster, 1998).

Pyrolysis (Py) methodology thermally cracks volatile organic compounds to gasify solid analytes. Samples are placed inside a small glass tube within a Pyroprobe. The Pyroprobe then inserted into an oven heated at, for this experiment, 610°C. The exposure to intense heat in an oxygen-free environment thermochemically breaks down complex molecules into more readily analyzable fragments; extreme heat breaks down plant compounds in preparation for gas chromatography. Pyrolysed analytes, through vaporization, become gas; the gas is then automatically injected in the GC for further separation and later the MS for identification of compounds within the analyte. (See Appendix D.)

Gas Chromatography (GC) separates volatile organic compounds in a gaseous or liquid analyte. First, the analyte is injected into a narrow tube known as a column using a microsyringe. GC instruments automatically inject samples (as opposed to the formerly used manual injection) to ensure greater precision and accuracy for both original and replicating research. After injection, the sample is heated to put analytes into a gas phase. The analyte is pushed through a silica column using helium as a carrier gas. Although hydrogen is the most efficient carrier gas for separation, helium is the most commonly used gas because it is non-flammable (unlike highly combustible hydrogen) and because it has comparable efficiency to hydrogen for most experiments. The compounds interact with the silica column, which determines how quickly the compound moves through the column. (See Appendix D.) Finally, compounds are graphically represented on an adjacent computer by a series of peaks. The peaks represent the amount of time it takes for each compound to travel through the column, referred to as retention time (Figure 1). Each compound travels at a different rate based on its physical properties; thus, the retention time helps identify compounds on the resulting chromatograph (Yang et al., 2005).

Mass spectrometry (MS) identifies compounds by measuring the mass-to-charge ratio (m/z) of ionized compounds in an analyte. Once the GC injects the sample into the MS, the analyte

is ionized to form both positively and negatively charged particles. The analyte enters the ion source where the components of the sample are converted to gaseous ions through massive collision with highly-charged electrons (Skoog et al., 1998). The resulting positive (most common) and negative gaseous ions are then streamed and accelerated into the mass analyzer. In the mass analyzer, electromagnetic fields then separate the gaseous ions based on their m/z. Once separated, ions are detected and graphically represented on an adjacent computer as mass spectra, indicating the quantity and abundance of each ion present in the sample. The vertical axis of the chromatograph can be in either parts per million (ppm) or in relative abundance. (See Appendix D.) For this research, relative percentage was used to more readily determine the preservation of potential biomarkers (compounds pre-selected as standards) among samples from different ages and different locations.

Once Py-GC-MS methodology has been implemented, chromatograms generated by the computer are analyzed. The horizontal axis of the chromatograph indicates time in minutes. This refers to how long it takes for each compound to run through the machine. Time is thus a measurement of mass-to-charge (m/z) ratio, the factor that determines how long it takes for elements and compounds to pass through the MS detector Lighter, less complex compounds like polysaccharide will be detected first, while complex compounds like fatty acids will show at the end of the chromatograph. Thus, retention time is not directly significant; instead, the inference that heavier, more complicated molecules will come at later times in the spectrograph is significant. To identify compounds, m/z in the mass spectra are compared to already known, studied, and tested compounds (called standards) from the National Institute of Standards and Technology (NIST). The 1996 NIST database contained over 75,000 spectra and continues to expand as new compounds are observed (McMaster and McMaster, 1998).

As a unified technique, the three components dramatically increased the quality of compound identification for both modern and fossil plant material. When combined, Py-GC-MS reduce the potential error found in any individual machine because each has different weaknesses and strengths; the likeliness of a compound eluding detection in each piece of equipment becomes highly unlikely when the three work in conjunction (Gudzinowicz et al., 1977). Py-GC-MS is the most effective and efficient form of compound identification, making it one of the most important methodologies in paleobiology research.

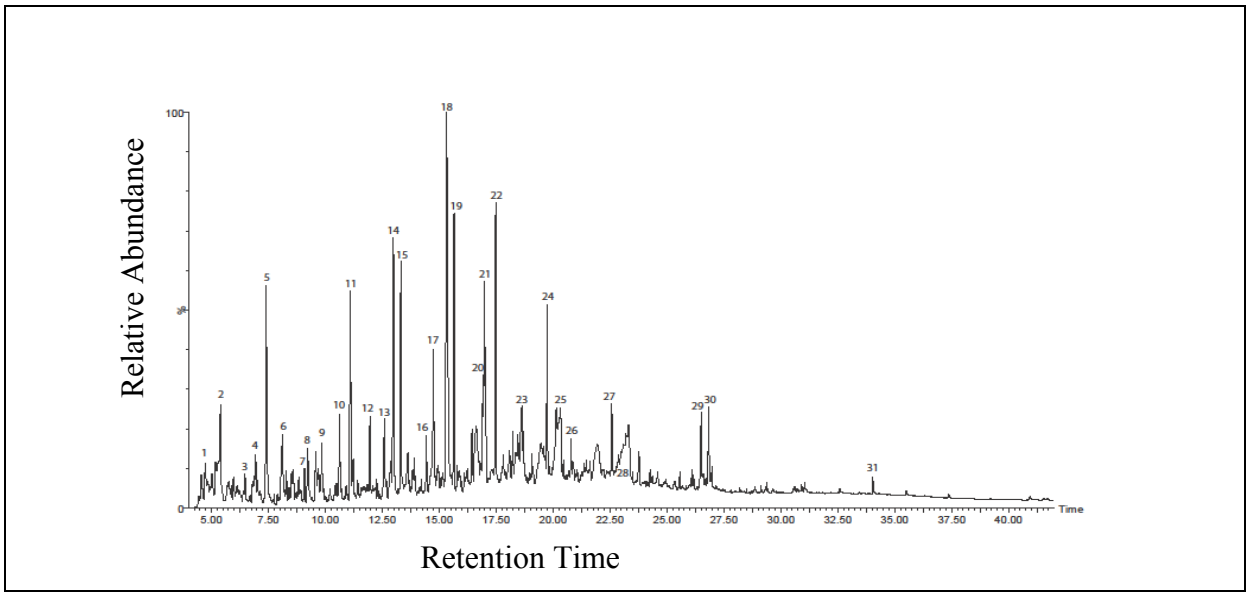


Figure 1 - Example of MS graph, Relative Abundance by Retention Time

Py-GC-MS technology and methodology was used to analyze the biomolecular composition of the fossilized conifer samples. CDS 5150 Pyroprobe at Massachusetts Institute of Technology (M.I.T.) was applied to expose samples to 610 °C in order to decompose compounds into smaller molecules for GC-MS analysis. Compound detection and identification were performed using on line Hewlett Packard HP6890 GC-MS in full scan mode under conditions explained in Gupta et al (2009). Compounds generated are identified using the US National Bureau of Standards mass spectral library and by comparing the compound spectra with previously papers that used the same method (Gupta et al., 2006; Yang et al., 2005; Ralph and Hatfield, 1991).

RESULTS

Solvent-extracted residues from Middle Miocene and Pliocene samples yielded abundant polysaccharide pyrolysis products such as 2-furaldehyde and levoglucosan, indicating excellent molecular preservation. In a modern conifer, typically the first third of an MS spectrum shows polysaccharides, the middle third shows a mix between lignin and polysaccharides, and the final third shows fatty acids (Yang et al. 2007). However, as samples increase in age, the amount of polysaccharide available in a sample generally decreases. The samples we observed remarkably have an abundance of polysaccharides that remained intact. The preservation of polysaccharides is a reflection of good preservation. In addition, these conifers fossils lack the commonly-gained lignin dimers at the end of the chromatograph. Lignin dimers are small, equally-distributed peaks that appear in poorly preserved conifers; the samples lack lignin dimers, indicating further excellent preservation. (See Appendix E.) These deposits thus demonstrate that the Banks Island materials possess high-quality molecular preservation equivalent to other Arctic *lagerstätten* and better than the Clarkia deposit.

The bulk peat exhibited a grouping of polysaccharides within the first ten minutes and a grouping of predominantly lignin with some polysaccharide in the middle of the chromatogram, suggesting the presence of conifer leaves within the peat. The Pliocene bulk peat from the Ballast Brook Formation on Banks Island was comparatively analyzed against all conifer samples taken from the Arctic region to determine the most abundant trees in the region. In comparison of bulk peat to other known conifers in the region, the bulk peat chromatogram (both in types and the abundance of compounds) appeared most closely related to *Picea*. After identifying MS compounds for the pyrolysates of both the Pliocene bulk peat (B7) and the Pliocene *Picea* (B6) best matched, the bulk peat displayed 87 percent likeness to *Picea* compound composition. This is significant in reconstructing the paleoenvironment. Figure 3-A and Figure 3-B respectively illustrates the total ion chromatograms (TIC) for the pyrolysates of the Pliocene *Picea* leaves and Pliocene bulk peat from Banks Island, Canada. The compound analyses of Py-GC-MS technology provide the relative intensity of detected compounds, as well as allots for detailed comparison between samples. The MS

identifications of the individual compounds, characteristic fragment ions, and suspected origin are presented in Tables 2 and 3.

Analysis of stable compounds offers potential as biomarkers; ratios of these compounds may function as standards for measuring plant fossil preservation. Using relative abundance ratios of 4-ethylphenol (m/z 122+107) or guaiacyl (m/z 109+124) with vinyl phenol (m/z 91+120) and levoglucosan (m/z 60+73) as indications for the preservation of lignin, cutin, and cellulose, we rank molecular preservation of these *lagerstätten* and compare these chemical data with SEM observations. All four potential biomarkers were analyzed in samples of *Glyptostrobus*, *Larix*, and *Picea* within their own genus over several geological epochs; Figure 4 demonstrates the relative abundance of guaiacyl, vinyl phenol, and levoglucosan between *Glyptostrobus* and *Larix* throughout modern day, the Miocene, and the Eocene.

Analysis of *Larix* and *Glyptostrobus* across different geological ages (Paleocene, Eocene, Miocene, and Modern) and geographic locations suggests that the age of the sample does not correlate with its molecular preservation. Intra-genus variation of pyrolysates among conifers is attributed to different original molecular constitution and paleoenvironmental conditions for preservation. Our data provide molecular level assessment of plant fossil preservation at these high latitudinal sites and offer further information on the role of labile biomolecules in the preservation of three-dimensionally preserved morphological structures in these Arctic plant *lagerstätten*.

Py-GC-MS Compound Identifications

Time	Ref	Compound Name	Fragments (m/z)	Origin
5.04	1	Acetic acid	60, 43, 45	Polysaccharide
7.27	3	Toluene	91, 92, 65	Lignin
8.00	4	2-Furaldehyde	95, 96, 39	Polysaccharide
9.11	6	C2-benzene	91, 106, 105	Lignin
10.5	8	5-Methyl-2-furfuraldehyde	110, 109, 53	Polysaccharide(Ce)
10.94	9	Phenol	94, 66	Lignin
12.44	10	2-Methylphenol	108, 107, 79	Lignin
12.84	11	3-+4-Methylphenol (Co-eluting)	108, 107, 79	Lignin
13.17	12	2-Methoxyphenol (Guaiacol)	109, 124	Lignin
14.30	14	4-Ethylphenol	122, 107	Lignin
15.21	16	4-Methyl-2-methoxyphenol	138, 123, 95	Lignin
15.48	17	4-Ethenylphenol (Vinylphenol)	120, 91	Lignin
16.78	19	3-Methyl-1,2-benzenedoil	124, 78	Lignin
17.35	23	4-Ethenyl-2-methoxyphenol	150, 135	Lignin
18.12	24	2- Methoxy-4-(1-propenyl)phenol	164, 149, 77	Lignin
18.55	26	Vanillin	152, 109	Lignin
19.62	27	2-Methoxy-4-(2-(E)-propenyl)phenol	164, 149, 77	Lignin
19.99	29	Levoglucosan	60, 73	Polysaccharide(Ce)
20.65	32	Dihydroconiferyl alcohol	137, 182	Lignin
23.54	33	2-Methoxy-4-(2-(E)-propenal)phenol	178, 147, 135	Lignin
24.06	34	Unknown	56, 57, 55	Unknown

Table 2 - Pliocene Picea

Above is the MS compound identification for pyrolysates in Pliocene Picea (B6). References numbers correspond to Figure 3 - A. Abbreviations: Time - Retention time; Ref - Reference number that corresponds with Figure ; m/z – mass-to-charge ratio of characteristic fragment ions (Ralph and Hatfield, 1991; Yang et al., 2005;Logan, 1992; Huang et al., 1998).

Time	Ref	Compound Name	Fragments (m/z)	Origin
5.05	1	Acetic acid	60, 43, 45	Polysaccharide
7.30	3	Toluene	91, 92, 65	Lignin
8.02	4	2-Furaldehyde	95, 96, 39	Polysaccharide
10.52	8	5-Methyl-2-furfuraldehyde	110, 109, 53	Polysaccharide(Ce)
10.98	9	Phenol	94, 66	Lignin
12.48	10	2-Methylphenol	108, 107, 79	Lignin
12.87	11	3-+4-Methylphenol (Co-eluting)	108, 107, 79	Lignin
13.21	12	2-Methoxyphenol (Guaiacol)	109, 124	Lignin
14.33	14	4-Ethylphenol	122, 107	Lignin
15.24	16	4-Methyl-2-methoxyphenol	138, 123, 95	Lignin
15.49	18	5-Hydroxymethyl-2-furaldehyde	97, 126	Polysaccharide
16.81	20	4-Ethyl-2-methoxyphenol	152, 137	Lignin
17.39	23	4-Ethenyl-2-methoxyphenol	150, 135	Lignin
18.14	24	2- Methoxy-4-(1-propenyl)phenol	164, 149, 77	Lignin
18.62	26	Vanillin	152, 109	Lignin
19.65	27	2-Methoxy-4-(2-(E)-propenyl)phenol	164, 149, 77	Lignin
20.44	29	Levoglucosan	60, 73	Polysaccharide(Ce)
21.53	30	Acetovanillone	151, 166	Lignin
22.47	32	Dihydroconiferyl alcohol	137, 182	Lignin
23.58	33	2-Methoxy-4-(2-(E)-propenal)phenol	178, 147, 135	Lignin

Table 3 - Pliocene Bulk Peat

Above is the MS compound identification for pyrolysates in Bulk Peat (B7). Reference numbers correspond to Figure 3 – B. Abbreviations: Time - Retention time; Ref - Reference number that corresponds with Figure ; m/z – mass-to-charge ratio of characteristic fragment ions(Ralph and Hatfield, 1991; Yang et al., 2005;Logan, 1992; Huang et al., 1998).

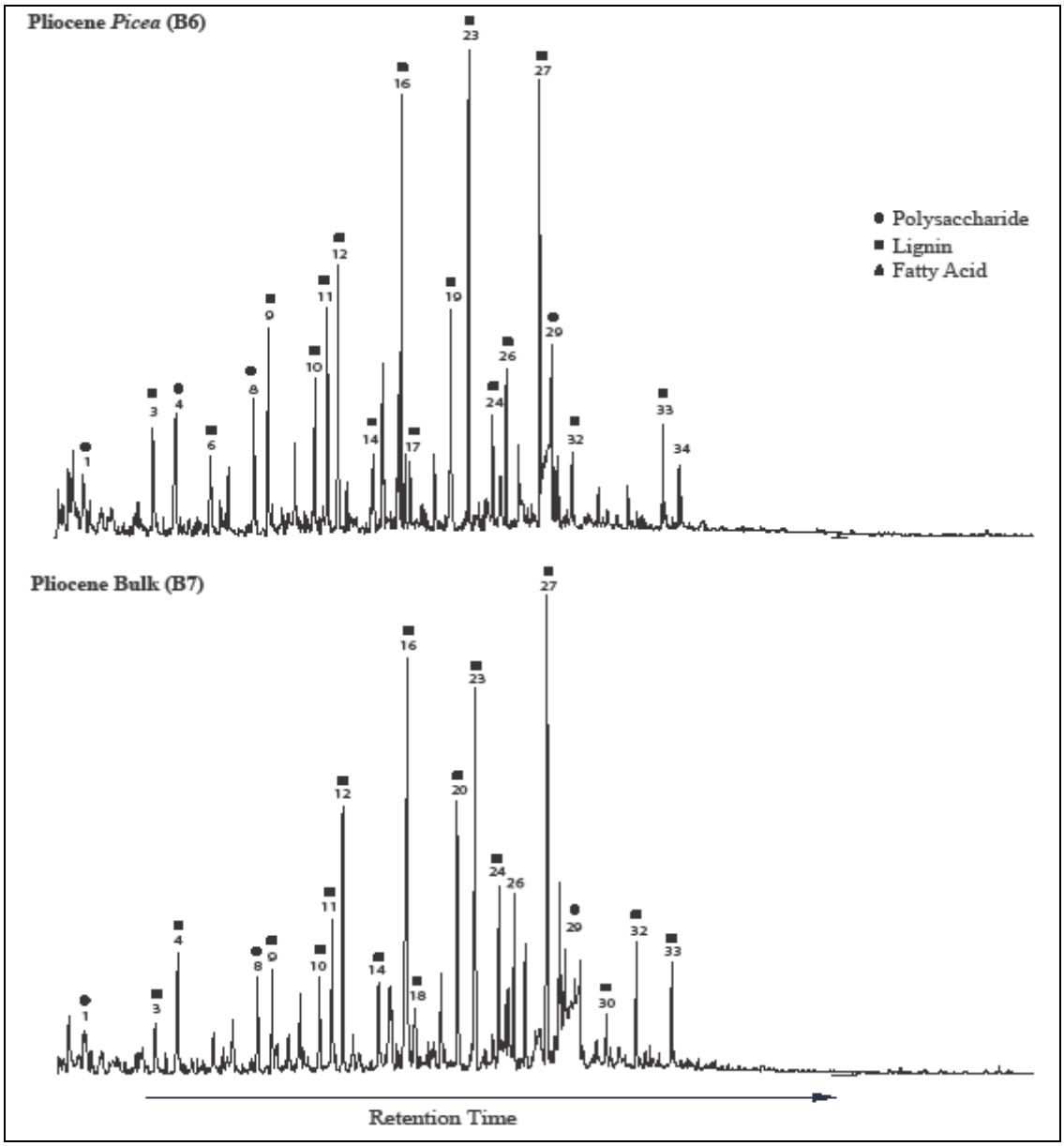


Figure 3 – Ion chromatograms for Pliocene Picea and Bulk Peat

Above are ion chromatograms of the pyrolysates showing the relative intensity of the pyrolysis products in percentage (vertical axis) obtained from A) Pliocene Picea leaves -B6, and B) bulk peat B7 from Banks Island, Canada for time in minutes (horizontal axis). Peak numbers correspond with MS identifications from Table 2 (Picea) and from Table 3 (Bulk Peat).

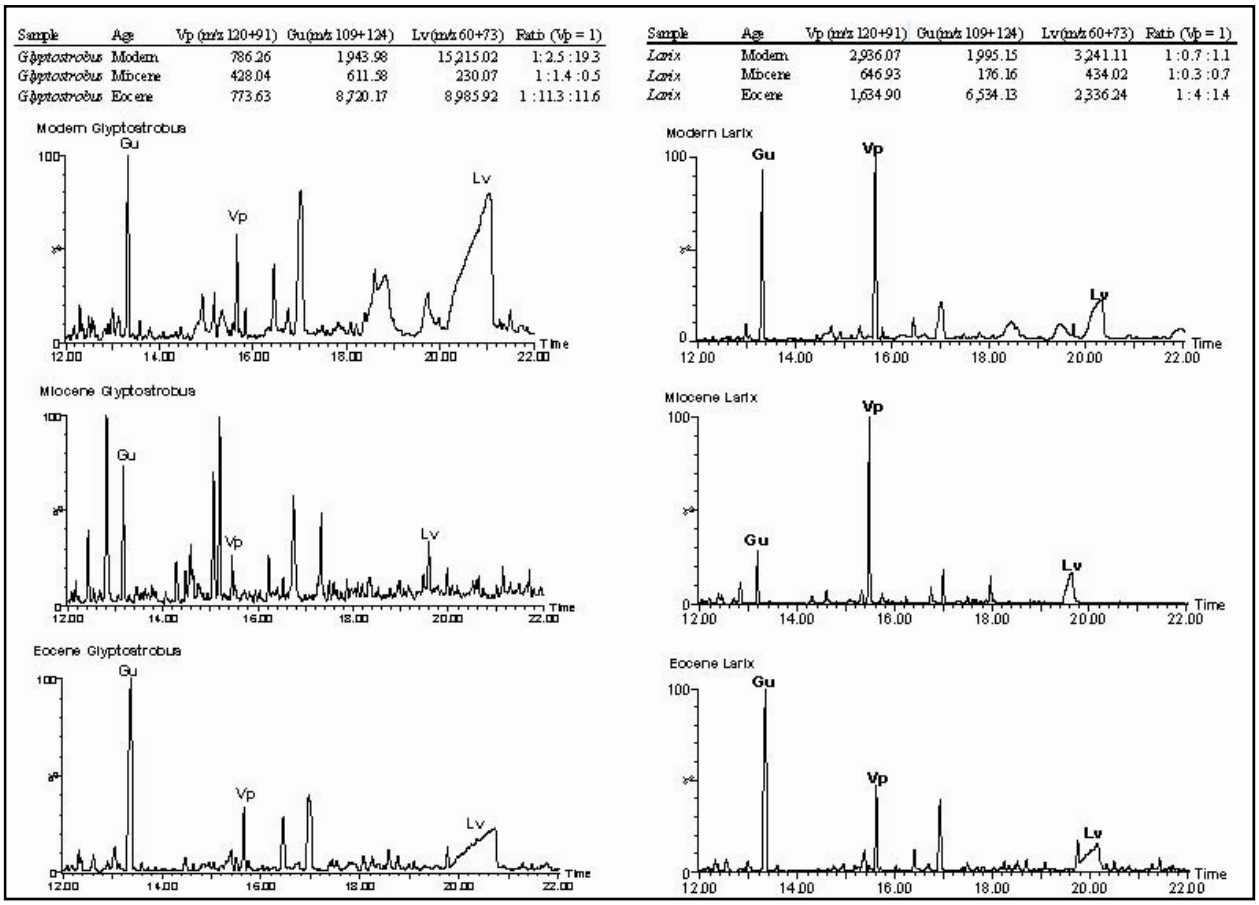


Figure 4 – Relative Abundance Ratios

Above are relative abundance of 4-ethylphenol (m/z 122+107), vinyl phenol (m/z 91+120), and levoglucosan (m/z 60+73) as indications for the preservation of lignin, cutin, and cellulose in percent (vertical axis) over time in minutes (horizontal axis); ratios of the relative abundance is displayed in the text charts on the upper left (Glyptostrobus) and the upper right (Larix). Graphs on the left represent Glyptostrobus and the graphs on the right represent Larix, both of which are in increasing order of age.

DISCUSSION

Both Py-GC-MS and SEM evidence indicate that fossil conifers found in Miocene and Pliocene deposits in the Banks Island (Williams et al., 2008) have excellent preservation on both molecular and morphological levels. The quality of these Banks Island fossils are at least equivalent to fossil conifers found in the Paleocene-Eocene deposits of the Axel Heiberg Island (Yang et al., 2005). In addition, comparison with fossil conifers from *lagerstätten* from Ellesmere Island, Canada (Paleocene-Eocene), Axel Heiberg Island, Canada (middle Eocene), and Clarkia, Idaho, USA (middle Miocene) deposits demonstrated that the Banks Island materials possess high-quality molecular preservation that is equivalent to other Arctic *lagerstätten* and is better than the Clarkia Miocene deposit. The exceptional molecular preservation warrants further molecular level isotope analysis using these Arctic fossil materials.

Analysis of *Larix* and *Glyptostrobus* fossilized leaves across different geological ages (Paleocene, Eocene, Miocene, and Modern) suggests that the age of the sample does not correlate with its molecular preservation. Better preservation in the Eocene over the Miocene and Pliocene samples diffuses possible correlation between age and preservation. Instead, intra-genus variation of pyrolysates among conifers is attributed to different original molecular constitution and paleoenvironmental conditions for preservation. Comparison of *Larix*, *Glyptostrobus*, and *Picea* across different geological ages also suggests that the preservation of the conifer fossil leaves is dependent on the paleoenvironment.

Solvent-extracted residues from *Larix*, *Glyptostrobus*, and *Picea* from a Middle Miocene site and *Picea* from a Pliocene site yielded abundant polysaccharide pyrolysis products such as 2-furaldehyde (also known as guaiacyl) from lignin and levoglucosan from cellulose, indicating excellent molecular preservation. However, the Eocene *Glyptostrobus* and Eocene *Larix* show even more remarkable preservation. Both Eocene sites indicate an incredible abundance of GC-MS peaks, including the three clusters frequently found their modern equivalents: polysaccharides in the first third, a polysaccharide-lignin mixture in the second third, and no trail of long-chain homologous pairs of aliphatic n-alk-1-enes/n-alkanes that is frequently found in fossilized conifers and associated with deterioration (Yang et al, 2005).

Comparison between pyrolysates of individual plants with Pliocene bulk material from the same deposit on from Banks Island, Northwest Territories, Canada revealed the dominance of conifers among these high latitude floras. Further analysis between the bulk peat and three conifers (*Larix*, *Glyptostrobus*, *and Picea*) reveal the predominance of *Picea* in the sample of bulk peat, as seen in their organic composition in Figure 1. The high percentage of similarity, approximately eighty-seven percent, in pyrolysis products between Pliocene peat sample and *Picea* suggests the dominating *Picea* is the source of vegetation for the Pliocene peat deposit in Banks Island.

Recent analysis of decayed conifer leaf tissues suggested that the ratio between vinyl phenol (m/z 91+120) and levoglucosan (m/z 60+73) can be used for monitoring the progress of preferential leaf tissue decay. According to Gupta 2009, vinyl phenol remains relatively constant over time because it is a stable lignin, whereas levoglucosan decreases over time because it is an unstable polysaccharide. Given the stability of vinyl phenol and the steady decline of levoglucosan over time, the preservation of a fossil could be easily and readily measured by the ratio of the two biomarkers compared to known preservation ratios. However, as seen in Figure 4, analysis of *Larix* and *Glyptostrobus* fossilized leaves across different geological ages (Paleocene, Eocene, Miocene, and Modern) with different levels of molecular preservation using biomarkers for lignin, cutin, and cellulose revealed the challenges of using this indicator. We suggest that intra-genus variation of pyrolysates among conifers is attributed to different original molecular constitution, rates of deterioration for each species, paleoenvironmental conditions for preservation, and age.

ACKNOWLEDGEMENTS

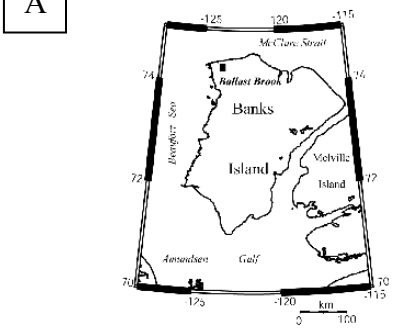
This work was partially supported by a NASA Rhode Island Space Grant and Bryant University Summer Research Fund. I would like to thank Christopher Williams for providing samples and diagrams (Appendix A), and Qin Leng for providing SEM observations. Thank you to Hong Yang for mentoring me throughout my research.

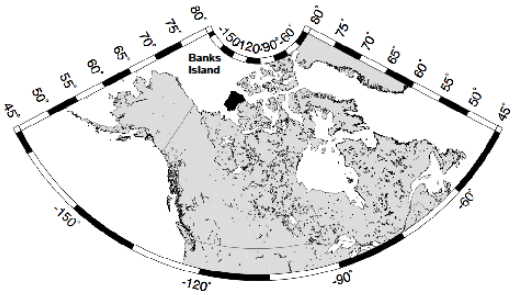
APPENDICES

Appendix A – Sample Location

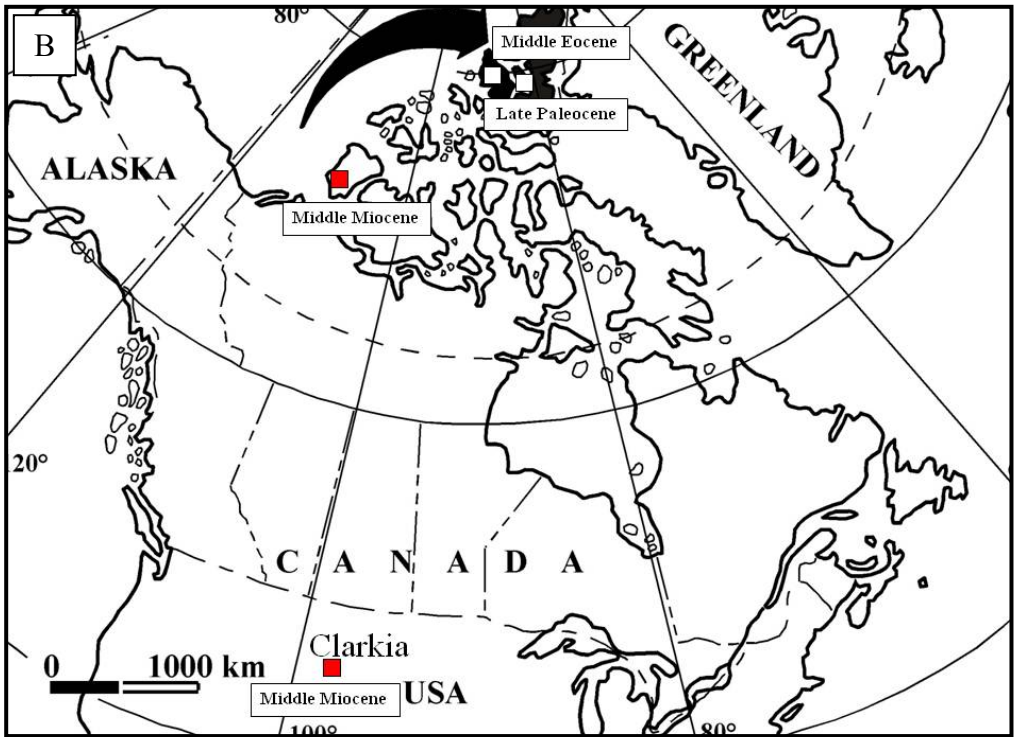
A, an enlarged view of the Canadian Arctic Archipelago, illustrates the position of fossil sites on Banks Islands, Northwest Territories, Canada. B, a view of North America, shows the three compared fossil sites: Clarkia, Idaho, USA; Banks Island, Northwest Territories, Canada; and Axel Heiberg Island, Nunavut, Canada (Yang et al., 2005; Williams et al., 2008).

A



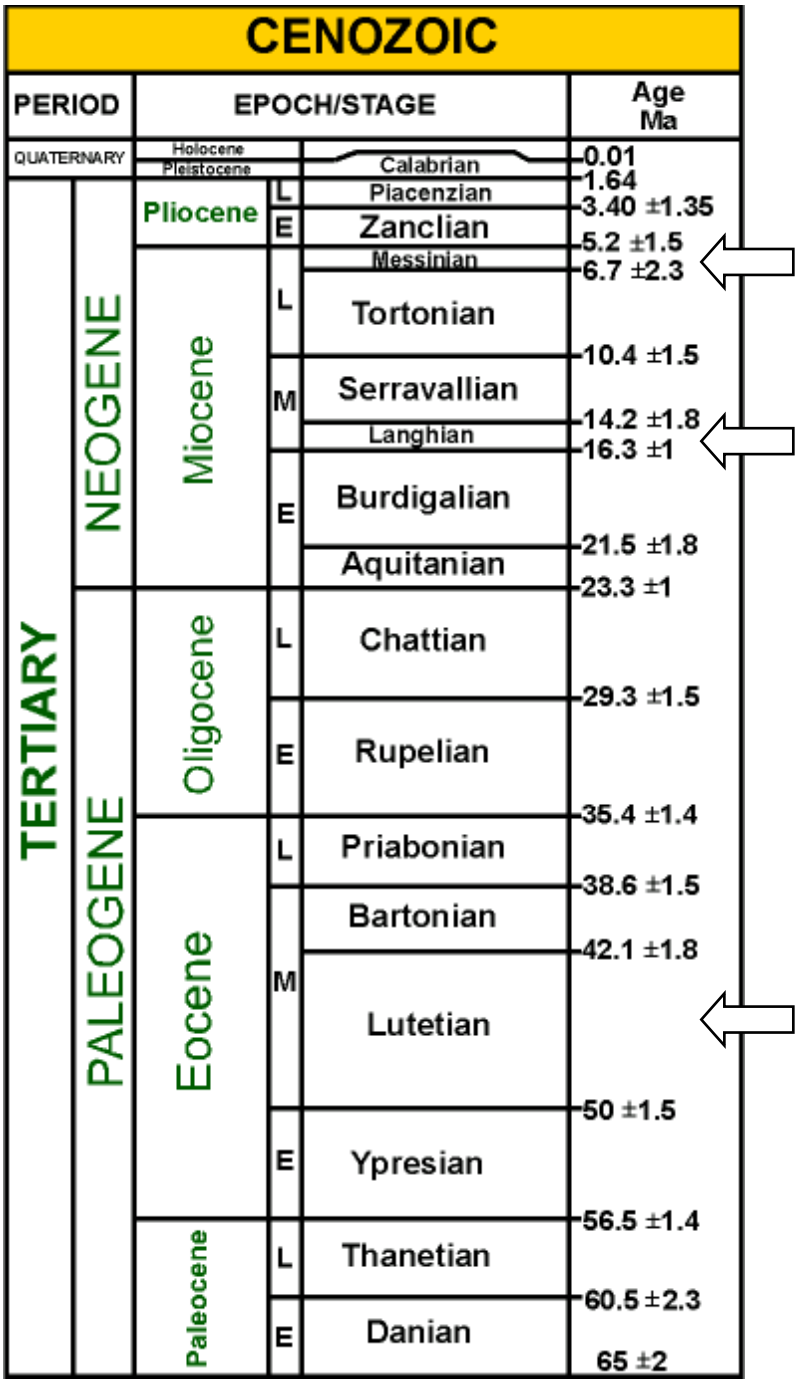


B

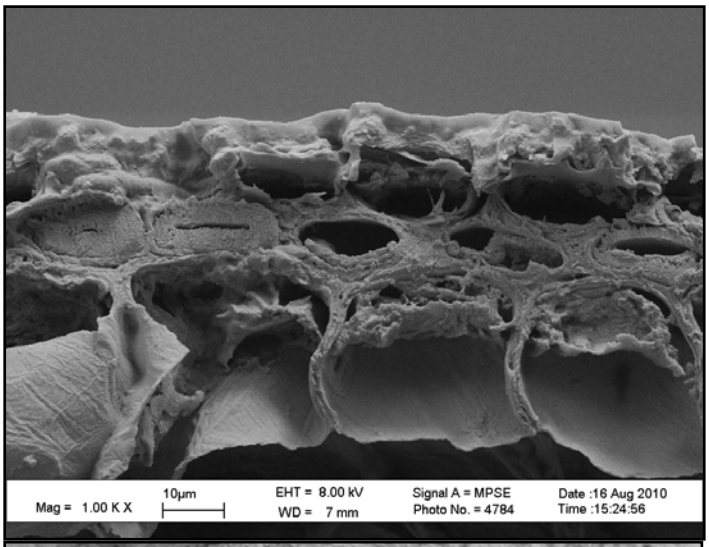


Appendix B – Geological Time Scale for the Cenozoic

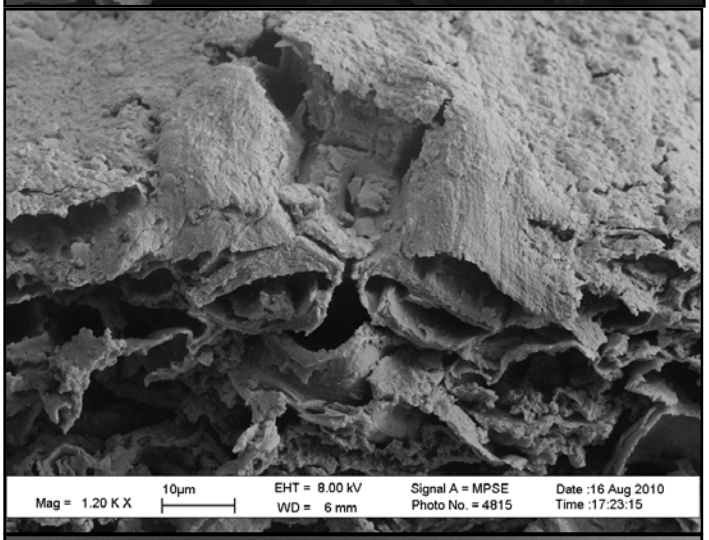
Arrows indicate approximate time periods studied: Pliocene (5 million years ago), Miocene (15 million years ago), and Eocene (45 million years ago) (PDAC, 1999).



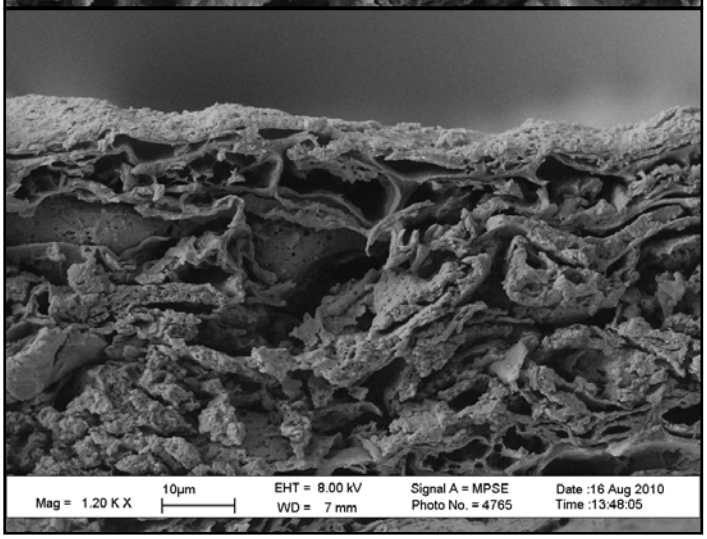
Appendix C- SEM Observations



A - Cross section of modern *Picea*, showing the enrichment of polysaccharides in epidermis cells



B - Cross section of Pliocene *Picea*, showing well preserved cuticle and the enrichment of polysaccharides near epidermis cells

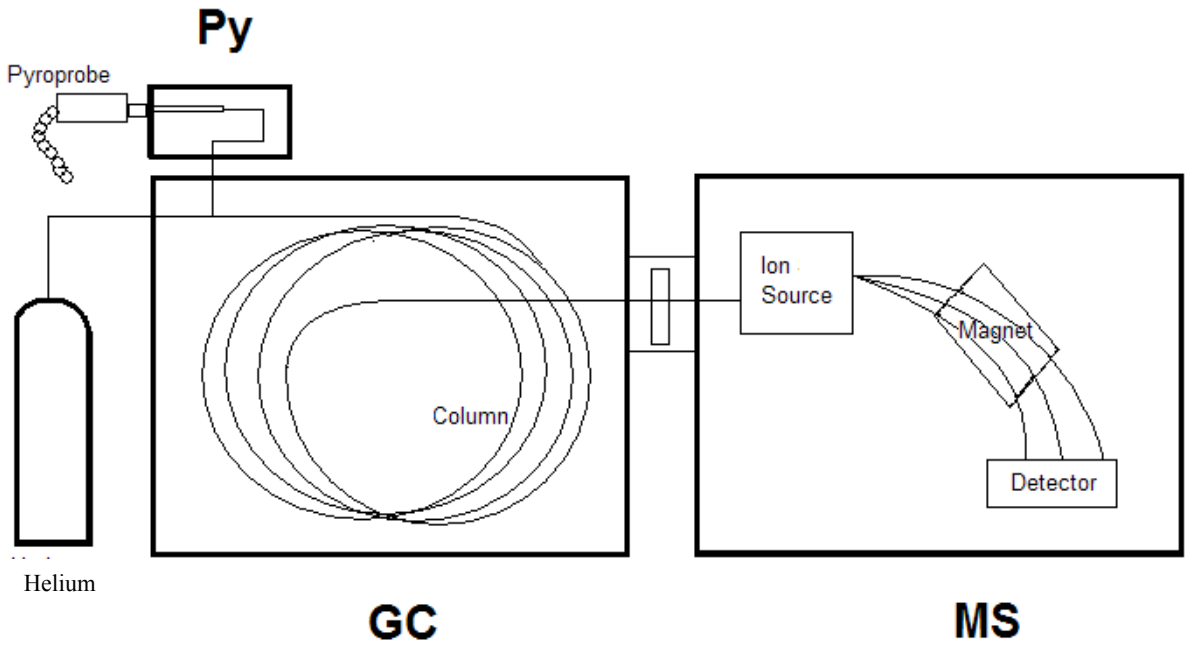


C - Cross section of Miocene *Larix*, showing excellently preserved cuticle and cell structures near the leaf margin.

All SEM illustrations were provided by Dr. Qin Leng.

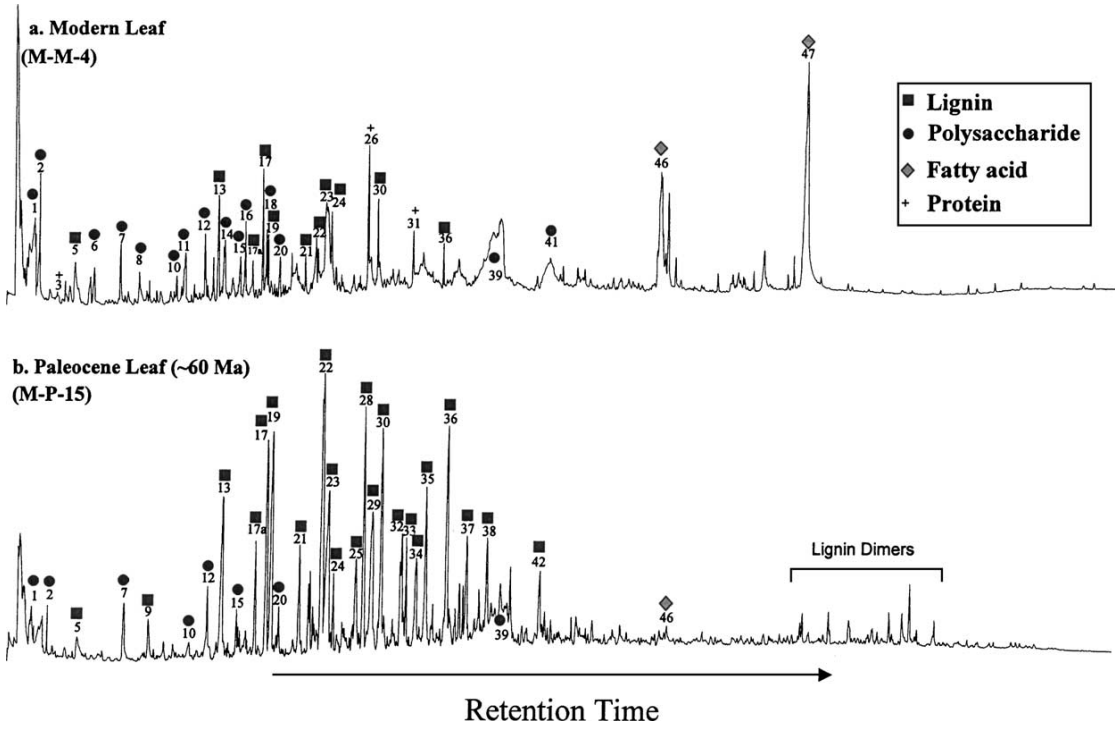
Appendix D – Py-GC-MS Technology

Below is a basic lay-out of Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS), starting with the analyte being inserted in the Pyroprobe to be decomposed through the Py. As the analyte is automatically injected into the GC, Helium (He) carrier is added to push the sample through the capillary column. Gas chromatography separates the compounds based on their volatiles. The analyte then passes through the flame detector and into the MS. Mass spectrometry takes the separated compounds from the gas chromatography and identifies each based on its mass-to-charge (m/z) ratio by first adding an ion source, passing the analyte through a magnet, and detecting the mass-to-charge ratios at the end detector. The detector displays results through an adjacent computer.



Appendix E – Example of Lignin Dimers

Lignin dimers appear at the end of a poorly-preserved sample, as seen in the Paleocene leaf compared to the modern leaf of Metasequoia (Yang et al., 2005).



REFERENCES

Basinger, J. F., 1991, The fossil forest of the Buchanan Lake Formation (early Tertiary), Axel Heiberg Island, Canadian Archipelago: preliminary floristics and paleoclimate: Geological Survey of Canada Bulletin, v. 403, p. 39-66.

Briggs, D.E.G., Evershed, R.P., Lockheart, M.J., 2000, The biomolecular paleontology of continental fossils, *in* Erwin, D.H. and Wing, S.L., eds., Deep time: Paleobiology's perspective: Paleobiology 26 (supplement to no. 4), p. 169-193.

Chaney, R. W., 1951, A revision of fossil *Sequoia* and *Taxodium* in western north America based on the recent discovery of *Metasequoia*: Transaction of the American Philosophical Society: New Series, v. 40, p. 171-239.

Eglinton, G., Logan, G.A., 1991, Molecular preservation: Philosophical Transactions of the Royal Society of London B333, p. 315– 328.

Fyles, J.G., Hills, L.V., Matthews Jr., J.V., Barendregt, R., Baker, J., Irving, E., Jette, H., 1994, Ballast Brook and Beaufort Formations (Late Tertiary) on Northern Banks Island, Arctic Canada: Quaternary International, v. 22/23, p. 141–171.

Gudzinowicz, B., Gudzinowics, M. J., Martin, H. F., 1977, Fundamentals of integrated GC-MS: M. Dekker, New York. p. 34-82.

Gupta, S., Yang, H., Leng, Q., Briggs, D. E. G., Cody, G. D., Summons R. E., 2009, Diagenesis of plant biopolymers: Decay and macromolecular preservation of *Metasequoia*: Elsevier., v. 40, p. 802-809.

Gupta, S., Briggs, D. E. G., Collinsond, M. E., Eversheda, R. P., Michelse, R., Jackf, K. S., and Pancost, R. D., 2006, Evidence for the *in situ* polymerization of labile aliphatic organic compounds during the preservation of fossil leaves: Implications of organic matter preservation: Elsevier, v. 38, p.499-522.

Huang, Y., Eglinton, G., van Der Hage, E. R. E., Boon, J. J., Bol, R., Ineson, P., 1998, Dissolved organic matter and its parent organic matter in grass upland soil horizons studied by analytical pyrolysis techniques: European Journal of Soil Science, v. 49, p. 1-15.

Jahren, H. A., and Sternberg, L.S. L., 2003, Humidity estimate for the middle Eocene Arctic rain forest: Geology, v. 31, p. 463-466.

Leng, Q., Langlois, G., and Yang, H., 2010, Early Paleogene Arctic terrestrial ecosystems affected by the change of polar hydrology under global warming: Implications for modern climate change in high latitudes: SciChina Earth Sci, doi: 10.1007/s11430-010-3081-5.

LePage, B. A., 2001, New species of *Picea* A. Dietrich (Pinaceae) from the middle Eocene of Axel Heiberg Island, Arctic Canada: Botanical Journal of the Linnean Society, v. 135, p. 137-167.

LePage, B. A. and Basinger, J. F., 1991, Early Tertiary Larix from the Buchanan Lake Formation, Canadian Archipelago, and a consideration of the phytogeography of the genus: Geological Survey of Canada Bulletin, v. 403, p. 67-82.

Liu, Y., Li, C., Wang, Y., 1999, Studies on fossil Metasequoia from north-east China and their taxonomic implications: Botanical Journal of the Linnean Society, v. 130, p. 267-297.

Logan, G. A., 1992, Biogeochemistry of the Miocene Lacustrine Deposit, Clarkia, Northern Idaho, USA, PhD thesis, University of Bristol, Bristol, England.

McIntyre, D. J., 1991, Pollen and spore flora of an Eocene forest, eastern Axel Heiberg Island, N.W.T.: Geological Survey of Canada Bulletin, v. 403, p. 83-98.

McIver, E. E., and Basinger, J. F., 1999, Early Tertiary floral evolution in the Canadian high Arctic: Annals of the Missouri Botanical Garden, v. 86, p. 523-545.

McMaster, M., and McMaster, C., 1998, GC/MS: a practical user's guide: Wiley-VCH, New York, p. 3-120.

Prospectors & Developers Association of Canada (PDAC), 1999, Geological Time Scale: http://www.northernminer.com/resources/timescl.pdf.

Ralph, J. and Hatfield, R. D., 1991, Pyrolysis-GC-MS Characterization of Forage Material: J. Agric. Food Chem., v. 39, p. 1426-1437.

Ricketts, B. D., and Stephenson, R. A., 1994, The demise of Sverdrup Basin: Late Cretaceous-Paleogene sequence stratigraphy and forward modeling: Journal of Sedimentary Research, v. B64, p.516-530.

Schweitzer, J., 2010, Scanning Electron Microscope, *in* Radiological & Environmental Management, http://www.purdue.edu/rem/rs/sem.htm.

Scoog, D. A., Holler, F. J., Nieman, T. A., 1998, Principles of Instrument Analysis: Saunders College Publishing: Philadelphia, PA, p. 255-256.

Smiley, C. J., and Rember, W. C., 1985, Physical setting of the Miocene Clarkia lake fossil beds, northern Idaho: Late Cenozoic History of the Pacific Northwest, Pacific Division of American Association for the Advancement of Science, San Francisco, CA, p. 11-30.

Smiley, C. J., Gray, J., Huggins, L. M., 1975, Preservation of Miocene fossils in unoxidized lake deposits, Clarkia, Idaho: Journal of Paleontology, v. 49, p. 833-844.

Stankiewicz, B.A., Briggs, D.E.G., Evershed, R.P., Flannery, M.B., Wuttke, M., 1997, Preservation of chitin in 25-million-year-old fossils: Science, v. 276, p.1541–1543.

Williams, C. J., Mendell, E. K., Murphy, J., Court, W. M., Johnson, A. H., Richter, S. L., 2008, Paleoenvironmental reconstruction of a Middle Miocene forest from the western Canadian Arctic: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 261, p. 160–176.

Williams, C. J., Johnson, A. H., LePage, B. A., Vann, D. R., Sweda, T., 2003, Reconstruction of the Tertiary *Metasequoia* forest: II. Structure, biomass, and productivity of the Eocene floodplain forests in the Canadian Arctic: Paleobiology, v. 29, p. 271-292.

Yang, H., and Leng, Q., 2009, Molecular hydrogen isotope analysis of living and fossil plants –*Metasequoia* as an example: Progress in Natural Science, v. 19, p. 901-912

Yang, H., and Hickey, L., 2006, *Metasequoia*: Back from the Brink? An Update: Bulletin of the Peabody Museum of Natural History, v. 48 (2), p. 317–327.

Yang, H., Huang, Y., Leng, Q., LePage, B.A., Williams, C.J., 2005, Biomolecular preservation of Tertiary *Metasequoia* Fossil *Lagerstätten* revealed by comparative pyrolysis analysis: Science, v. 134, p. 237-256.