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Lignin and Isotope Signatures in Pollen: A Caveat of Lignin Phenol Biomarker for Reconstructing Paleovegetation

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Abstract: Lignin phenol and stable isotope have been used as a biomarker to trace the sources of sedimentary organic matter (SOM), reconstruction of paleovegetation and climate changes without considering the possible occurrence of fossil pollen grain in sedimentary matrix. Lignin phenol analysis of modern pollen showed that both gymnosperm and angiosperm pollen yielded very high concentration of cinnamyl (C) phenols as compared to that of vanillyl (V) and syringyl (S) phenols (C/V = 3.05 – 23.67) with variation in isotopic compositions ($\delta^{13}C$ = –20.2 to –28.3‰ and $\delta^{15}N$ = –9.2 to 1.7‰). Therefore the isotopic and lignin phenol signatures in SOM might be distorted to some extent by fossil pollen. This was especially true in the sediments of low altitude and latitude continental lakes and coastal areas where significant amount of fossil pollen might be deposited. Therefore, before using lignin phenol as a biomarker, one should consider a possible distortion derived from lignin of fossil pollen in sedimentary matrix.

Key words: Lignin, pollen, vegetation, biomarker, biogeochemistry.

Introduction

Lignin and stable carbon isotope signatures in organic matter of lacustrine sedimentary deposits have a significant applicability for reconstructing paleovegetations (Leopold et al., 1982: Tareq et al, 2004, 2006; Castañeda et al., 2009), consequently regional and global paleoclimate. The applications of isotope and lignin phenols as source indicators of sedimentary organic matter (SOM) are based on data obtained from modern plants and have still several uncertainties. One of such uncertainties is an occurrence of fossil pollen in the lacustrine sedimentary deposits. Pollen grains were known to be most resistant to microbial degradation among the sedimentary organic matter, and thus might also be important organic carbon (OC) contributors to the total SOM (Keil et al., 1994; Hu et al., 1999). There was a considerable amount of pollen grains in lacustrine and coastal marine environments (Chmura et al., 1999; Vincens et al., 2005). A significant occurrence of pollen grain in sedimentary matrix of lacustrine and coastal marine environments might skew the results of isotope and lignin signatures in SOM. It is, therefore, necessary to improve our knowledge about the isotopic and chemical characteristics of pollen to enhance the reliability of stable isotope and lignin phenols as biomarkers.

Recently few studies focussed on stable carbon isotope analysis of pollen for different plants of diverse geographic environments (Amundson et al., 1997;

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Loader and Hemming, 2001, 2004; Jahren, 2004). Pollen can be isolated from the sedimentary matrixes using chemical treatment without damaging pollen tissues (Bennett and Willis, 2001), recommending pollen as an ideal plant tissue substrate for carbon isotope analysis as well as other terrestrial biomarkers, especially, lignin phenol analysis. Yet there was no systematic analysis for lignin phenol composition of pollen tissues (Hu et al., 1999; Ishiwatari et al., 2006).

This study sought to characterize the stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) and the CuO-oxidation products of lignin of modern pollen from representative samples of angiosperm and gymnosperm plants. The stable isotope values and CuO-oxidation products of lignin were compared to those of reported values for plant vegetative tissues (woody and nonwoody) to clarify a possible effect of fossil pollen tissues on isotopes and lignin phenol parameter values of SOM for reconstructing paleovegetation and to determine the suitability of pollen as a natural archive of these biomarkers. The compositional differences of these biomarkers between gymnosperm and angiosperm pollens are also discussed.

Materials and Methods

Sampling

Pollen samples were collected from Japan Forest Tree Breeding Center in Sendi, Japan. Pollen collection was performed mostly during late April and/or early May because the greatest numbers of species were actively producing pollen during this time in Japan. Samples were taken from 20 different plants grown in Japan. Seven of the collected samples were gymnosperm (conifer) plant pollen while rest of the 13 samples were angiosperm plant pollen (Table 1). The pollen samples in vials were kept frozen before analyses.

Elemental and Stable Isotope Analysis

Total organic carbon (TOC) and total nitrogen (TN) were measured using an elemental analyzer (Thermo Quest NA2500 NCS). Half of the samples (n = 10) run three times for estimating the reproducibility of the measurements, and the analytical deviation is less than ±3% for both TOC and TN. Stable carbon and nitrogen isotopic compositions of pollen samples were determined using an elemental analyzer interfaced, via a Finnigan CONFLO III system, to a mass spectrometer (Finnigan Delta Plus^{XP}). Data quality control throughout the analysis was ensured by running a reference standard after every three runs and cross check with international atomic energy agency (IAEA) sucrose standard (IAEA-CH-6, $\delta^{13}C = -10.43 \pm 0.1‰$). Average reproducibility based on replicate measurements for $\delta^{13}C$ and $\delta^{15}N$ were about 0.1 and 0.2‰, respectively.

Lignin Phenol Analysis

Lignin analysis was carried out according to the method developed by Hedges and Ertel (1982) and Goni and Hedges (1992) with minor modifications. Briefly, the procedure involved the addition of 15-20 mg of pollen sample, CuO (1 g) and $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$ (100 mg) to 5 ml of 2 N nitrogen-purged NaOH solution in a stainless steel bomb. The bomb was heated at 170 °C for 3 hrs on a digital shaker (KS501, IKA Labortechnik). A reaction mixture was acidified to pH 1, and lignin phenols were extracted with peroxide-free diethyl ether. The ether extract was dried over anhydrous Na_2SO_4 overnight and then concentrated under reduced pressure. Lignin phenols were converted to trimethylsilyl derivatives with bis(trimethylsilyl) trifluoroacetamide (BSTFA) and pyridine.

Quantitative analyses of lignin phenols were performed using a capillary column (J&W Scientific, DB1, 60 m × 0.25 mm i.d.) installed in a gas chromatograph (Shimadzu 14B GC). The GC conditions were as follows: injector temperature of 300°C, flame ionization detector temperature of 300°C, and oven temperature program started at 130°C with five minutes initial delay increased to 190°C at a rate of 3°C/min, 190°C to 200°C at a rate of 1°C/min and 200°C to 280°C at a rate of 4°C/min with a final hold time of 10 minutes. Lignin phenols were quantified using ethylvanillin as a GC internal standard and their individual response factors relative to a mixture of commercially available authentic standards.

The more resistant portion of pollen, hereafter called sporopollenin, was isolated from modern pollen by acetolysis according to the method developed by Bennett and Willis (2001). Briefly, pollen was treated with glacial acetic acid and a mixture of acetic anhydride and sulphuric acid (9:1) for few minutes in an ultrasonicator (Bransonic 220H®) at room temperature to hydrolyze and remove cellulose and other labile organic carbon. After centrifuging and decanting, glacial acetic acid was again added, centrifuged, and decanted, followed by multiple distilled water washes until odour of acetic acid was no longer evident. Samples were then oven dried and lignin phenol composition was measured by the method as described in the above section. The response factors of GC-FID relative to standard compounds were monitored weekly and did not vary significantly (±2%, n = 12) over the three months

of analysis period. The mean deviation of the analytical procedure for all lignin phenols was better than ±5% except for the p-hydroxyl phenols (±10%) for replicate (n = 3) analyses of some selected samples.

Results

Elemental and Stable Isotope Compositions of Modern Pollen

The results of OC, TN, $\delta^{13}C$ and $\delta^{15}N$ measurements of pollen samples are presented in Table 1 along with lignin phenol data. The OC and TN contents of different pollen species showed small scale variability. In general, gymnosperm pollens have slightly lower values for both of OC and TN than those of angiosperm pollens. The C/N values of both gymnosperm and angiosperm pollens varied within small ranges, and gymnosperm pollen has generally high value as compared to angiosperm pollen (Table 1). Both $\delta^{13}C$ and $\delta^{15}N$ values showed no systematic variations between gymnosperm and angiosperm taxon as well as different species of individual groups. The $\delta^{13}C$ values of pollens from both taxa were fallen within specified ranges representative for the C_3 plants ($\delta^{13}C$ of C_3 plants: –23 to –27‰) but values were slightly positive. The $\delta^{13}C$ and $\delta^{15}N$ values of both gymnosperm and angiosperm pollen showed weak correlation (Figure 1, r^2 = 0.35) and cannot be used as an end member for SOM sources.

Table 1: Elemental, isotopic and CuO-oxidation lignin phenol compositions of various pollen samples collected in Japan

Pollen name	*OC (%)*	*TN (%)*	*C/N*	*$\delta^{13}C$ (‰)*	*$\delta^{15}N$ (‰)*	*Vl mg/g*	*Sl mg/g*	*Cl mg/g*	*pH mg/g*
			Gymnosperm						
Pinus massoniana	49.7	2.2	22	-24.9	0.5	0.7	-	15.0	9.5
Pinus densiflora	44.0	2.2	20	-25.7	-0.7	0.7	-	13.9	12.1
Pinus thunbergii	49.3	2.4	21	-26.3	-3.2	0.6	-	14.3	11.0
Larix leptolepis	44.0	4.8	9	-23.3	-2.0	0.9	-	4.2	8.4
Cryptomeria japonica	44.3	1.9	24	-23.1	0.4	2.9	-	8.8	6.1
Chamaecyparis obtusa	44.1	2.2	20	-21.2	-1.2	1.6	-	6.6	5.2
Picea glehnii	47.4	3.9	12	-20.2	1.2	1.2	-	17.4	16.0
Average	46.1	2.8	18	-23.5	-0.7	1.2	-	11.5	9.8
±SD	±2.6	± 1.1	±5	±2.3	±1.5	±0.8		±4.9	±3.7
			Angiosperm						
Betula platyphylla	48.1	3.6	13	-28.1	-9.2	3.6	0.4	4.0	6.2
Betula ermani	49.9	4.7	11	-26.5	-5.6	3.9	0.9	4.5	7.4
Betula davurica	50.1	4.2	12	-25.8	-6.2	3.6	1.0	4.2	6.1
Betula maxmowiczianaa	49.9	4.4	11	-26.8	-5.0	4.7	1.6	5.7	10.8
Betula lutea var. allebh.	50.5	4.4	11	-26.6	-4.8	4.0	1.4	4.7	10.1
Betula ovalifolia	51.1	4.6	11	-26.7	-4.0	4.9	0.9	5.6	11.7
Alnus japonica	54.2	4.1	13	-28.3	-6.1	2.3	0.7	5.9	13.3
Alnus trabeculosa	55.5	3.7	15	-26.1	-5.9	2.0	0.5	4.6	14.3
Alnus fauriei	51.8	5.0	10	-25.0	-4.9	1.8	0.4	4.4	19.2
Quercus crispula	51.7	6.2	8	-24.5	-1.1	2.7	1.1	5.2	12.2
Quercus serrata	50.9	6.4	8	-27.0	-1.6	3.1	0.4	5.0	9.5
Quercus acutissima	56.6	5.1	11	-27.1	1.7	2.8	0.4	4.7	9.6
Quercus aliena	52.3	7.0	8	-27.0	-4.6	2.6	0.4	4.5	11.0
Average	51.7±	4.9	11	-26.6	-4.4	3.2	0.8	4.8	10.9
±SD	2.4	±1.1	±2	±1.1	±2.7	±1.0	±0.4	±0.6	±3.5

Abbreviations: OC: organic carbon; TN: total nitrogen; Vl: total vanillyl phenols; Sl: total syringyl phenols; Cl: total cinnamyl phenols; pH: total p-hydroxy phenols.

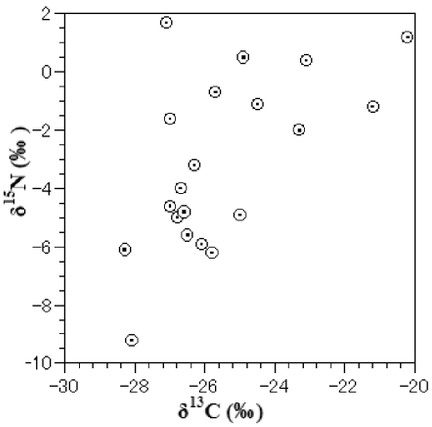

Figure 1: Relationship between $\delta^{13}C$ and $\delta^{15}N$ values of gymnosperm pollen (GP) and angiosperm pollen (AP).

Alkaline CuO-oxidation Products of Modern Pollen

Alkaline CuO-oxidation of pollen samples yielded a suite of lignin derived phenols comprising vanillyl (V), syringyl (S), cinnamyl (C) and p-hydroxyl (pH) phenols (Table 1). To clarify the characteristics of alkaline CuO-oxidation products of modern pollens, their lignin phenol properties (defined as parameters in Table 2) are compared with fresh plant lignin phenol data from literatures (Figure 2). Among CuO-oxidation products of lignin, the yields of V-phenols from the gymnosperm pollen were much lower than those from needles, a vegetative tissue, and no appreciable amount of S-phenols was produced from the pollens of gymnosperm like their vegetative-tissues (Figure 2a). In contrast to gymnosperm pollen, relatively elevated V-phenols and trace amount of S-phenols were found in the angiosperm pollens. Pollens contained much lower concentrations of V- and S-phenols as compared to the respective vegetative-tissues (woody and non-woody) in both gymnosperm and angiosperm.

Table 2: Definition of different lignin phenol parameters

Parameter	*Definition*
λ_8	∑8 Lignin phenols of vanillyl, syringyl, cinnamyl (mg/100 mg OC)
V	∑3 Vanillyl phenols (mg/100 mg OC)
S	∑3 Syringyl phenols (mg/100 mg OC)
C	∑2 Cinnamyl phenols (mg/100 mg OC)
S/V	Ratio of total syringyl to total vanillyl
C/V	Ratio of total cinnamyl to total vanillyl
(Ad/Al)v	Ratio of vanillic acid to vanillin
(Ad/Al)s	Ratio of syringic acid to syringaldehyde

The yields of C-phenols of pollen from gymnosperm plant were much higher than those of non-woody gymnosperm vegetative-tissues while C-phenols of pollen from angiosperm plant were comparable to non-woody angiosperm vegetative-tissues (Figure 2a). The elevated yields of C-phenols from the pollens of both taxa were caused almost exclusively by high p-coumaric acid contents, and yields of the ferulic acid from the pollen samples were very low or undetectable (data not shown). Gymnosperm pollens yielded elevated amount of C-phenols compared with those of angiosperm pollens, and pollen from gymnosperm plants, *pinus* and *picea*, contained particularly high C-phenols (Table 1). The total yields of eight lignin phenols (λ_8) normalized to TOC from gymnosperm and angiosperm pollens were smaller than those of the respective woody tissues and comparable to non-woody vegetative-tissues (Figure 2a).

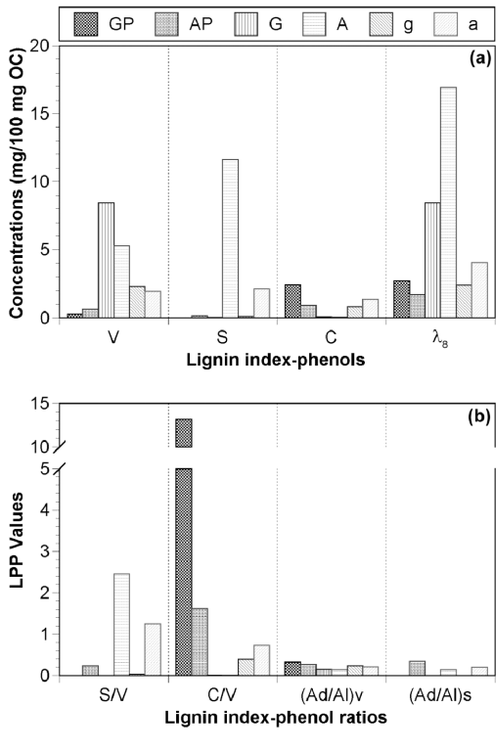

Figure 2: Comparison of lignin phenol parameters (LPP: *see* Table 2 for definition) in modern gymnosperm pollen (GP) and angiosperm pollen (AP) and fresh gymnosperms woody (G), angiosperm woody (A), gymnosperm non-woody (g) and angiosperm non-woody (a) plant tissues of various species. (a) lignin derived index-phenols and (b) lignin derived index-phenol ratios. Fresh plant lignin-phenol data obtained from literatures (Hedges and Mann 1979; Hedges et al., 1985; Ertel and Hedges, 1985; Alberts et al., 1991; Goni and Hedges, 1992) and a few number of plants analyzed in the present study.

The S/V values of gymnosperm pollen were zero like their vegetative-tissues, whereas the S/V values of angiosperm pollens ranged from 0.12 to 0.42 with an average value of 0.24 ± 0.10 (n = 13). The values were very low as compared to the values of their woody tissues and non-woody vegetative-tissues (Figure 2b). The C/V values of pollens from gymnosperm and angiosperm plants ranged from 3.05 to 23.67 with an average of 13.16 ± 9.04 (n = 7) and from 1.11 to 2.52 with an average of 1.63 ± 0.52 (n = 13), respectively. The C/V showed high value for gymnosperm pollen as compared to angiosperm pollen. Among gymnosperm pollen, genus *pines* and *picea* had extremely high C/V values, 21 and 24, respectively.

The acid:aldehyde ratios of V-phenol [(Ad/Al)v] of gymnosperm and angiosperm pollens ranged from 0.10 to 0.53 with an average value of 0.30 ± 0.13 and from 0.10 to 0.45 with an average value of 0.26 ± 0.10, respectively. The Ad/Al values of S-phenol [(Ad/Al)s] of angiosperm pollens ranged from 0.09 to 0.49 with an average value of 0.32 ± 0.11. The Ad/Al values of pollen from both taxa were slightly higher than those of corresponding woody and non-woody vegetative-tissues (0.10 to 0.30) but much lower than diagenetically alternated lignin materials (1.0 to 3.0) found in natural environments (Figure 2b). Thus, the Ad/Al values of pollens might not overprint the diagenetic state inferred by these parameters of sedimentary lignin phenols.

Alkaline CuO-oxidation Products of Sporopollenin

Fossil pollen has been subjected to diagenetic processes and the sporopollenin, the most resistant portions of pollen, persists in long geological records (Hemsley et al., 1992). Sporopollenin was isolated from the modern pollen to determine lignin compositions of more resistant fraction of pollen. Results of lignin phenol analyses of the isolated sporopollenin showed that the concentrations of V- and S-phenols in sporopollenin of gymnosperm and angiosperm pollens were comparable to those of corresponding modern pollens except C-phenols which were more concentrated in sporopollenin (Figure 3a, b). The elevated yields of C-phenols from the sporopollenin of both taxa were also caused almost exclusively by high yield of p-coumaric acid, and the ferulic acid was undetectable in these sporopollenin samples. The values of lignin phenol parameters (*see* definition in Table 2) of sporopollenin and pollen were significantly correlated (r^2 = 0.98, p < 0.001; Figure 4).

The three p-hydroxy (pH) lignin phenols, p-hydroxybenzaldehyde, p-hydroxyacetophenone and p-hydroxybenzoic acid, were common oxidation products of pollen from both taxa (Table 1). Half of the CuO-oxidation products of modern pollen were pH-phenols. The abundances of pH-phenols were

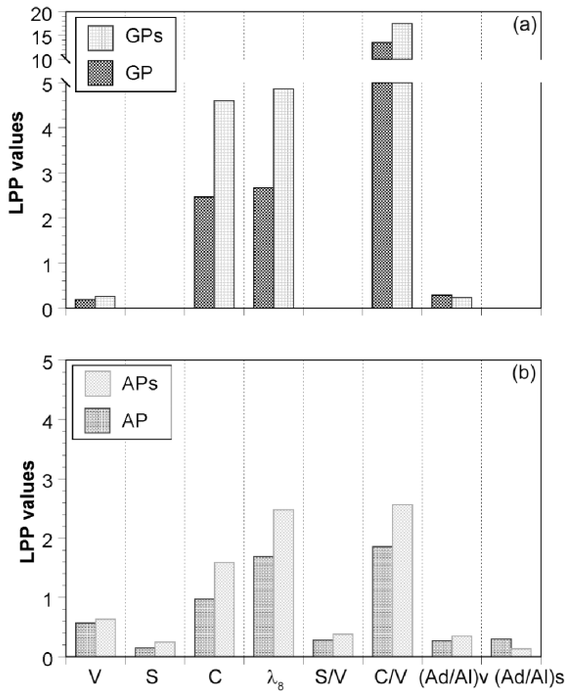

Figure 3: Comparison of lignin phenol parameters between (LPP) (a) modern gymnosperm pollen (GP) and chemically isolated sporopollenin from modern gymnosperm pollen (GPs) and (b) modern angiosperm pollen (AP) and chemically isolated sporopollenin from modern angiosperm pollen (APs). Abbreviations were explained in Figure 2 and text.

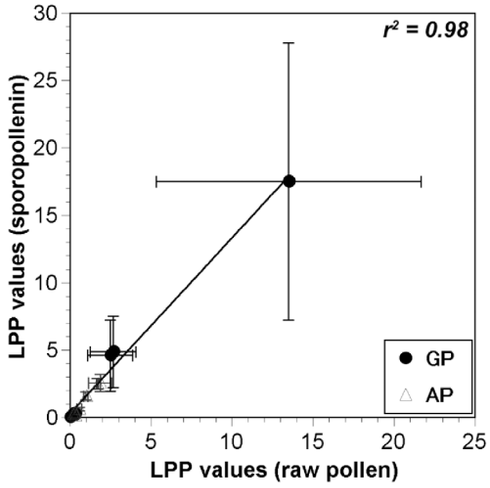

Figure 4: Relationship of lignin phenol parameters (LPP) between modern pollen and chemically isolated sporopollenin. Abbreviations: GP: gymnosperm pollen, AP: angiosperm pollen.

greater than those of total S- and V-phenols, and less than or comparable to C-phenols, although abundances of these compounds were variable in different species. The elevated yield and close correspondence between concentrations of pH-phenols and C-phenols indicated that these two groups of phenols were characteristic of lignin polymer present in pollen tissues.

Lignin Phenol Vegetation Index (LPVI)

The LPVI values were calculated according to the definition in Tareq et al. (2004) [LPVI = $\{S(S+1)/(V+1)+1\}\times\{C(C+1)/(V+1)+1\}$]. The LPVI values of gymnosperm and angiosperm pollens ranged from 225 to 1841 with an average of 1039 ± 714 and from 96 to 720 with an average of 307 ± 188, respectively. LPVI values of gymnosperm pollens were extremely higher than those of the gymnosperm woody tissues (G) and non-woody vegetative-tissues (g), while the LPVI values of angiosperm pollens were slightly higher than those of angiosperm woody tissues (A) and were very low as compared to that of angiosperm non-woody vegetative-tissues (a) (Figure 5). The LPVI values of sporopollenin from both taxa, i.e., gymnosperm and angiosperm pollens, ranged from 616 to 2119 with an average value of 1370 ± 751 and from 673 to 924 with an average value of 783 ± 128, respectively. These values were comparable to those of respective modern pollen but showed little higher values (Figure 5).

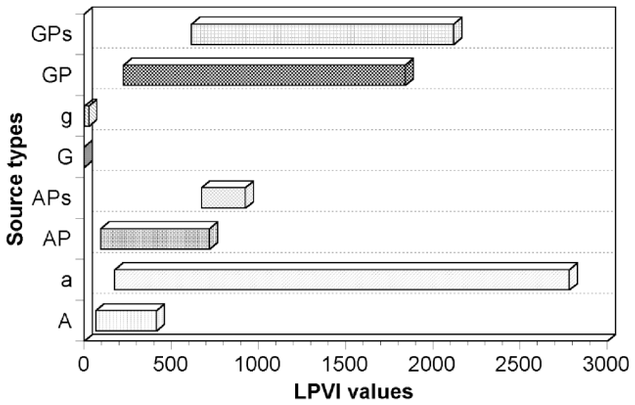

Figure 5: Comparison of lignin phenol vegetation index (LPVI) of chemically isolated sporopollenin from modern gymnosperm pollen (GPs), modern gymnosperm pollen (GP), fresh gymnosperm non-woody (g) and gymnosperms woody (G) plant tissues, as well as chemically isolated sporopollenin from modern angiosperm pollen (APs), modern angiosperm pollen (AP), fresh angiosperm non-woody (a) and angiosperm woody (A) plant tissues of various species. Fresh plant lignin-phenol data obtained from literatures (Hedges and Mann, 1979; Hedges et al., 1985; Ertel and Hedges, 1985; Alberts et al., 1991; Goni and Hedges, 1992) and a few number of plants analyzed in the present study.

Discussion

Elemental and Stable Isotope Signatures in Pollen

The small differences in OC and TN contents between gymnosperm and angiosperm pollens can be attributed to physiological nature and complex distributions of different organic compounds (cellulose, lignin, lipids and protein) in pollen tissues of different species and/or other environmental factors. Pollen is composed of approximately 2-10% cellulose, 2-24% of sporopollenin and a complex biopolymer of carotenoids and their esters (Brooks and Shaw, 1971). Different proportions of these pollen components in each species are probably responsible for the OC and TN values observed for pollen in the present study (Table 1).

From the studies of tree-ring and $\delta^{13}C$ values (Schleser, 1994) and blooming period temperature of *Pinus sylvestris* pollen and $\delta^{13}C$ values (Loader and Hemming, 2001), it was known that $\delta^{13}C$ value was closely correlated with ambient temperature and such relationship might be particularly explained as a relative change in stomatal conductance resulting from a variation in the temperature during blooming periods. It was true that $\delta^{13}C$ values and pollen blooming period temperature showed a positive relationship in the present study (Figure 6), however, the correlation coefficient and slope of regression line were lower than those found from the pollen of *Pinus sylvestris* (Loader and Hemming, 2001).

The strong positive correlation between $\delta^{13}C$ value and blooming period temperature of pollen reported by Loader and Hemming (2001) was obtained from a single species collected from Europe. In the present study, pollen specimens were collected from various taxa and locations. Thus, the relationship between $\delta^{13}C$ value and blooming period temperature was not so strong as indicated by large number (n = 176) of various pollen samples collected across the United States (Jahren, 2004). The present and previous observations suggested that the blooming period temperature was one of the key factors controlling $\delta^{13}C$ values of pollen, but other factors, e.g., species characteristics and environmental conditions other than blooming period temperature (e.g., seasonal light intensity and relative humidity), might be also important, although the present study was not able to distinguish the biological and environmental effects on the $\delta^{13}C$ value of pollens.

Caveats for Lignin Phenol Biomarker

The most striking feature of the lignin phenol composition of pollen from gymnosperm and angiosperm plants was that the C-phenols were yielded in high concentration compared with those of V-phenols, subsequently extremely high C/V values were obtained from pollen specimens of both taxa (Figure 2a, b). The high abundance of p-coumaric acid has also been documented in the sporopollenin skeleton of pollen (e.g., *pinus mugo*) by pyrolysis mass spectrometry (Wehling et al., 1989). The C-phenols were the important source related signature for non-woody plants, especially C/V (see Table 2). The sporopollenin, the most resistant portions of pollen also showed higher values of lignin phenol parameters than those of modern pollen, especially high C/V values due to the presence of huge amount of C-phenols (Figure 3). Previous studies have used the C/V values of fresh leaves/needles as a geochemical end member of non-woody vascular plant tissue to estimate the contribution of non-woody vegetative-tissue in SOM of lacustrine and marine environments without taking pollen into consideration (Hedges et al., 1982; Leopold et al., 1982; Yamamoto et al., 2005; Tareq et al., 2004, 2006). Such estimates were reasonable if pollen concentrations were low in sediments, but such interpretation might be biased by pollen-derived lignin phenol signal, if significant amount of pollen had been deposited in the sedimentary matrix.

The pH-phenols were well known CuO-oxidation products of lignin in both gymnosperm and angiosperm plant vegetative-tissues (Sarkanen and Ludwig, 1971; Hedges and Parker, 1976). While non-vascular plants (Hedges and Parker, 1976) and some other biochemical precursors (e.g., tyrosine) also yielded pH-phenols (Hedges et al., 1988; Goñi and Hedges, 1995) upon CuO-oxidations. Due to such ambiguous nature, pH-phenols of CuO-oxidation products were not included as a lignin phenol parameters in the previous studies (Leopold et al., 1982; Tareq et al., 2004, 2006). However, CuO-oxidation products of both modern pollen and sporopollenin contained a significant amount of pH-phenols like C-phenols. Thus, the levels of pH-phenols should be considered during the measurement of the lignin composition of SOM to evaluate the effect of deposited pollen grain to the sedimentary lignin signatures.

Both modern pollen and sporopollenin of the gymnosperm and angiosperm showed a wide range of values for LPVI (Figure 5). Since the LPVI values of both modern pollen and sporopollenin of angiosperm were within the LPVI ranges of angiosperm woody tissues and non-woody vegetative-tissues, the sedimentary angiosperm pollen grain derived lignin could not seriously bias the vegetation information provided by LPVI. However, if excessive amount of angiosperm pollen grains deposited in sedimentary matrix as compared to plant vegetative-tissue, LPVI might include the contribution of non-woody plants.

The high LPVI values of gymnosperm pollens as compared to both woody tissues and non-woody vegetative-tissues of gymnosperm plant suggested that gymnosperm pollen might seriously bias the vegetation information provided by LPVI. Some of the gymnosperm plants, e.g., pine, produced excessive amount of pollen grain as compared to other terrestrial plants (Miltner et al., 2005; references therein). Presence of sustainable amount of gymnosperm pollen grain in sedimentary matrix could change LPVI inferences from gymnosperm to angiosperm plants (woody and non-woody) (Figure 6). Therefore, before applying LPVI to reconstruct vegetation, one should consider a possible distortion derived from lignin of fossil gymnosperm pollen in sedimentary matrix.

High contribution of lignin from fossil pollen in sedimentary matrix can be detected from the lignin phenol parameter values as discussed above, especially high yields of C-phenols, subsequent extremely high C/V values. The C-phenols derived from both modern pollen and sporopollenin are mostly composed of p-coumaric acid, as the yields of ferulic acid were low

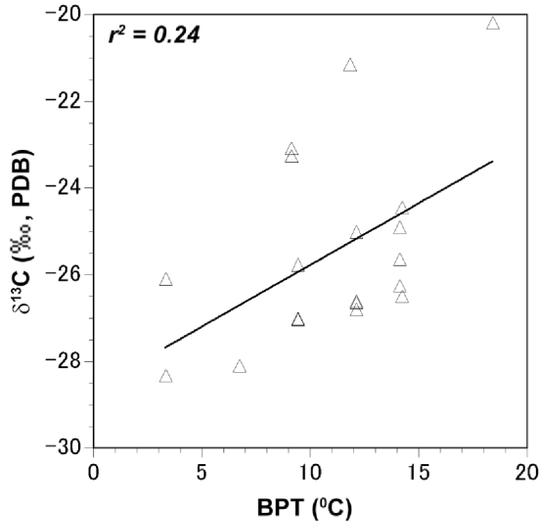

Figure 6: Relationship between pollen-δ13C values and blooming period temperature (BPT). BPT are average values of 1971-2000 at the nearest meteorological observatory which is appeared in the chronological scientific Tables 2004 edited by National Astronomical Observatory and published by Maruzen Co. Ltd., 2003.

or undetectable, while C-phenol from vegetative tissues are mostly composed of both p-coumaric and ferulic acid (Hedges and Mann, 1979; Alberts et al., 1991; Goni and Hedges, 1992). Thus, the high concentration of C-phenols, mostly comprised of p-coumaric acid, can qualitatively separate pollen derived lignin signals in SOM.

Conclusion

Results of the present study showed that the isotope and lignin phenol compositions of SOM might be changed by isotopic and lignin phenol signal of pollen. This was especially true on the sediments of low altitude and latitude continental lake and coastal sedimentary deposits, where significant amount of fossil pollen might be deposited. So, we should consider possible fossil pollen derived lignin contributions before using lignin phenol as a biomarker in SOM of lacustrine and coastal sedimentary deposits. Development of a reliable isolation method for sufficient amount of fossil pollen from sedimentary matrix for isotope and CuO-oxidation lignin phenol measurements may explore the applications of this natural archive for valuable new information on terrestrial environmental changes.

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