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Plant Polymer Palaeobiology- Aphidoidea (PL³-A) Project: Geochemical and Morphological Studies on Gall (-like) Fossils

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ABSTRACT

Pyrolytic analysis was performed on a leaf fossil, containing a gall-like spherule structure, from the Eocene Ikushunbetsu Formation in the Yubari area, Hokkaido, Japan, in order to examine the potential and applicability of the geochemical methods for identification of the trace fossils of insects such as gall. Several compounds such as *n*-alkanes and alkyl benzenes and naphthalenes were detected in leaf blade sample, whereas they were absent in the spherule sample. These results indicate that the gall-like spherule in the leaf fossil is neither an organic fossil nor a gall. However, as organic molecules could be detected in a small amount of leaf sample, the method used has potential to identify organic fossil originated from gall. Combination of morphological and geochemical studies on the gall fossils is suggested to provide essential information for understanding coevolutionary relationships between terrestrial higher plants and insects, at geologic time scale, as well as macroevolutionary patterns in plant - insect associations.

Keywords: Gall fossil, Plant - insect coevolution, Resistant macromolecule (polymer), Aphid (Aphidoidea)

INTRODUCTION

Coevolution has been suggested as the main driving force for the diversity of the associations between terrestrial higher plants and insects. Fossil records of the insect - plant associations possibly provide us important information for coevolutionary relationships between these organisms at geologic time scale as well as macroevolutionary pattern in these associations. Trace fossils of insects within plant tissues such as galls and leaf miners can be typical paleontological objects of insect - plant associations [1–5]. However, there have been few studies for the trace fossils of insects, because of few

collections and identifications. Table 1 shows a comprehensive list of previous studies on the Cenozoic gall fossils. Previous studies mainly reported gall mites (Acari: Eriophyoidea), gall midges (Diptera: Cecidomyiidae), and gall wasps (Hymenoptera: Cynipidae), as groups of gall inducers, from Europe or North America. This distribution pattern is incompatible with the present distribution of galling insects. Most of the fossil galls are leaves on which neither the structure of the gall nor its inducer has been preserved. The fossil record of plant galls is quite meager.

We have been systematically searching the trace fossils of insects, and studying them by not only

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Table 1 Fossil host plants and galling insects in the Cenozoic

Age	Fossil host plants	Galling insects	Formation#	Location	Ref.
Late Miocene	<i>Fagus pristina</i>	<i>Mikiola pontensis</i> (Diptera)	*No description	La Cerdaña basin, Spain	I
	<i>Fagus gussonii</i>	<i>Aceria nervisequa</i> (Acari)			
	<i>Quercus drymeja</i>	<i>Eriophyes vilarrubiae</i> (Acari)			
		<i>Contarinia</i> sp. (Diptera)			
		Hymenoptera			
	<i>Quercus hispanica</i>	<i>Neuroterus</i> (Hymenoptera)			
	<i>Persea</i> sp.	Acari			
	<i>Zelkova zelkovaefolia</i>	Acari			
	<i>Acer pyrenaicum</i>	<i>Artacris macrorhynchus</i> (Acari)			
		Acari			
	Magnoliophyta	Diptera			
Middle Miocene	<i>Quercus hannibali</i>	<i>Antronoides schorni</i> (Cynipid)	UCMP Locality PA428 horizon	Washoe County, Nevada, USA	II
Miocene	<i>Quercus simulata</i>	<i>Antronoides cyanomontanus</i> (Cynipid)	*Unknown	Oregon, USA	III
Eocene	Nothofagaceae	Acari	La Meseta Fm.	King George Island & Seymour Island, Antarctica	IV
		Diptera			
		Hemiptera			
		Hymenoptera			
Middle Pleistocene	<i>Distylium racemosum</i>	<i>Nipponaphis</i> sp. (Aphid)	Osaka Gr.	Hirotoyama, Nishinomiya City, Japan	V

References, I: Diéguez *et al.* [2], II: Waggoner and Poteet [3], III: Schick and Erwin [4], IV: McDonald *et al.* [5], and V: Tsukagoshi *et al.* [8]
#: Fm.: Formation, Gr.: Group.

morphological observations but also organic bio (geo) chemical analyses, in particular using plant macromolecules (polymers). Macromolecular analysis in combination with morphological analysis was recently developed and applied to plant and algal organic fossils [6, 7]. The trace fossils of insects can be morphologically observed and described by comparing them with extant galls and leaf miners. They often occur as spherules within leaf fossils and fragments, which may be difficult to identify as insect fossils. Organic bio (geo) chemical analyses are useful for identification of insects (and plants) occurring within such spherules and fragments of fossils. Furthermore, studies on physiological associations between plant and insect seem to be possible by a combined morphological and (geo) chemical approach.

Under the COE 'Plant Polymer Palaeobiology-Aphidoidea (PL³-A)' Project that began in 2006, we have mainly focussed on the Cenozoic gall (-like) fossils. We found some gall fossils, in Dr. S. Miki's plant fossil collections, which come from the Middle Pleistocene *Sapium* Bed in Hirotoyama of Nishinomiya City, Hyogo Prefecture, Japan. A gall fossil

that had been tentatively identified as *Distylium racemosum* was described in detail in [8] (Table 1). The fossil was most likely generated from aphidoidea, and moreover, from its specific genus *Nipponaphis* (Table 1).

Here, we present data based on pyrolytic analysis of the Eocene gall (-like) fossil in order to investigate organic molecules constituting the fossils, and examine the organic bio (geo) chemical methodology of such analysis for identification of gall fossils.

MATERIALS AND METHODS

Sample

A fossil leaf sample (Fig. 1a) is contained in sediment that formed a part of a drill core, obtained by the Hokkaido Colliery and Steam-ship Co. Ltd., from the Eocene Ikushunbetsu Formation in the Yubari area, central Hokkaido, Japan. This leaf fossil was tentatively identified as *Cercidiphyllum* sp. by Mr. H. Taniguchi, although its species is still unclear. There is a gall-like spherule structure on this leaf fossil as shown in Fig. 1a. The spherule was isolated from the leaf with tweezers and obtained as

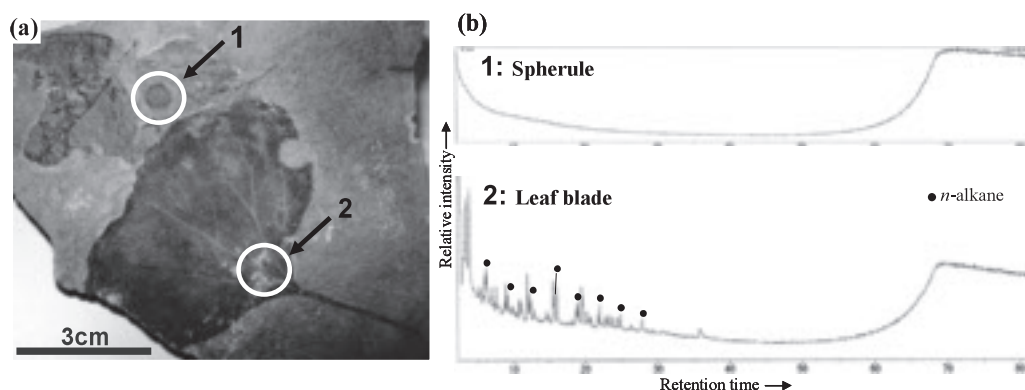


Fig. 1 (a) Photograph of the Eocene leaf fossil that contains gall-like spherule structure, (b) Total ion chromatograms (TICs) of pyrolysates in parts of the spherule (1) and the leaf blade (2) within a leaf fossil.

fine powder sample. In addition, the leaf tissue ('leaf blade' sample) was obtained as fine powder sample for comparison with the 'spherule' sample. These powder samples were injected into pyrolytic gas chromatography (GC) - mass spectrometer (MS) system, and analyzed for organic compounds generated from them.

Pyrolytic GC-MS

Pyrolytic analysis was performed on-line using a Shimadzu Pyr-4a pyrolysis unit at the Faculty of Science, Hokkaido University. Samples were weighted (ca. 100 μg) in a 3 mm x 3 mm platinum (Pt) bucket. The bucket was placed in the sample holder, which was affixed to the top of the Pyr-4a, for 5 min keeping the bucket at room temperature and under a He gas stream. The sample bucket was then dropped into the heated zone maintained isothermal at 450°C or 650°C. Identification of pyrolytic compounds was carried out by GC-MS with a Shimadzu GC17A attached to a capillary GC (60 m $\times$ 0.25 mm i. d. DB-5 column, J & W Scientific) directly coupled to a Shimadzu QP5000 quadrupole mass spectrometer (electron voltage, 70 eV; emission current, 350 μA ; mass range, m/z 50–550) at the Faculty of Science, Hokkaido University. The injector was maintained at 310°C and with a splitless. The GC temperature was programmed as follows: 80°C for 2 min, 80–120°C at 20°C/min, 120–310°C at 3°C/min, and 310°C for 20 min.

RESULTS AND DISCUSSION

The gall-like spherule structure on the leaf fossil from the Eocene Ikushunbetsu Formation is about 1.0 mm in diameter. The spherule structure is gray in color and is surrounded by light brown circular

area. From these morphological features, this structure was possibly induced by insects such as aphids on the leaf. Pyrolytic analyses under 450°C and 650°C temperature conditions were carried out on a leaf fossil containing a gall-like spherule structure, using GC-MS. As no compounds were detected by the pyrolytic analysis at 450°C, we infer that the organic matter of leaf fossil consists of refractory compounds that cannot be decomposed at low temperature condition. The total ion chromatograms (TICs) of the pyrolytic analyses at 650°C are shown in Fig. 1b. Short-chain *n*-alkanes and alkenes in which carbon number 7 (C_7) to C_{14} homologues are mainly detected in 'leaf blade' sample (spot 2 of Fig. 1a). These alkyl compounds might have been derived from resistant macromolecules such as cutin in leaf tissue of the fossil. Also, aromatic hydrocarbons such as alkyl benzenes and alkyl naphthalenes were identified in the leaf blade sample. These aromatic hydrocarbons are likely to be diagenetic products from phenolic compounds contained in living leaves. However, they were absent in 'spherule' sample (spot 1 in Fig. 1a). These data indicate that the gall-like spherule in the leaf fossil is neither an organic fossil nor a gall. The spherule structure observed might represent a mineral spherule formed inorganically in the leaf remain after deposition. However, as we could detect organic molecules by using a small amount of leaf sample, the method used has potential to identify organic fossils originated from galls.

In another study [9] of a PL³-A project, emphasis is given to the study of the phenolic compounds from resistant macromolecule using the pyrolytic analysis, especially of tannin and its monomer (s), which are generally peculiar and abundant in gall. Several gallic derivatives such as gallic acids could

be identified from a giant fossil gall induced by an aphid from the Middle Pleistocene *Sapium* Bed in Hirotayama. The gallic acid is generally known to be a typical monomer of hydrolysable tannin. Our finding that such gallic derivatives are well preserved in gall fossil points to their possible use for identification of gall type and its chemotaxonomy, although further examination is necessary. Hence, we will continue to explore and analyze gall samples and other trace fossils by insects using this method.

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