Heating experiments on Pinus pollen grains and its relation to petroleum genesis

YOSHIHIRO UJIÉ,* YUUKI ARATA** and MIYABI SUGAWARA

Department of Earth and Environmental Sciences, Hirosaki University, Bunkyo-chou 3, Hirosaki 036-8561, Japan

(Received May 17, 2002; Accepted January 24, 2003)

Spores and pollen are regarded as possible progenitors of petroleum. They are transformed into type II kerogen in sediments during diagenesis, which generates petroleum hydrocarbons on catagenesis and metagenesis. Living-pollen grains of Pinus thunbergii were heated to 290°C for 24 hours under the atmosphere in the laboratory to investigate the changes in physical and chemical properties of pollen with regards to the hydrocarbon generation. It was shown that heated pollen grains have decreasing trends in weight, size, atomic H/C ratio and stTAI, but an increasing trend in atomic N/C ratio with increasing temperature, especially in the higher temperature region. The changes in the atomic H/C ratio indicated that hydrocarbon generation from living-pollen grains should take place in the temperature range of about 135 and 230°C.

INTRODUCTION

Organic maturation, the chemical and physical processes of organic matter in sediments i.e., diagenesis, catagenesis and metagenesis, involves a complex of basically first-order chemical reactions depending primarily on temperature and duration of heating but little on pressure, and catalysis of minerals and organic matter (Philippi, 1965; Connan, 1974; Dow, 1977). Heating experiments or pyrolysis cannot be compared directly to subsurface organic maturation in sedimentary rocks. However they provide a simple convenient simulation of organic maturation. Heating experiments on organic matter in sediments have generally been conducted in closed systems under an inert atmosphere (nitrogen or helium). Heating experiments on living pollen and spores have been conducted in open air conditions (Gutjahr, 1966; Yule et al., 1999), as pressure greatly retards organic maturation of living pollen and spores (Sengupta, 1974). Physical and chemical features of living pollen and spores changed remarkably on heating to 250°C in the open air condition (Yule et al., 1999, 2000).

Sporopollenin, a highly resistant biomacromolecule in the outer wall of spores and pollen, can survive in sediments for over millions of years with full retention of morphology. However, their chemical and physical properties are modified by diagenesis, catagenesis and metagenesis after deposition. Among those, the color change of spore and pollen is recognized as one of the most useful parameters for organic maturation in the petroleum exploration.

Sporopollenin is generally made from macromolecules represented by general empirical formula of C₉₀H₁₂₄O₁₈, C₉₀H₁₃₄O₃₁, C₉₀H₁₅₈O₄₄ and so on (Zetzsche and Vicari, 1931; Brooks, 1971). Since 30–35% by weight of the original

*Corresponding author (e-mail: ujiie@cc.hirosaki-u.ac.jp)
**Present address: 2nd Network Engineering Division, NEC Communication Systems, Ltd., 1131 Hinode, Abiko, Chiba 270-1174, Japan
sporopollenin could be converted to oil after ozonization (Shaw and Yeadon, 1966), spore and pollen are also regarded as possible progenitors of petroleum. After sediments including spore and pollen are buried, they are most often transformed into type II kerogen, which can generate petroleum in sedimentary rocks during catagenesis and metagenesis. Type II kerogen is not excellent but rather a good source material to provide commercial oil (Tissot and Welte, 1984). Therefore fossil pollen and spores can be direct maturity indicators for petroleum genesis, and laboratory heating experiments on living pollen or spores may provide direct evidence for petroleum formation and organic maturation.

The pollen of *Pinus* sp. is most frequently found as fossils in Cretaceous to Recent sediments in Japan. In this study, living-pollen grains of *Pinus thunbergii* growing widely in the Honshu, Shikoku and Kyushu Islands, were heated to 290°C in laboratory and the changes in size, weight loss, atomic H/C and N/C ratios, and brightness were measured. The pollen of *Pinus thunbergii* is composed of 9.12% moisture, 4.46–4.91% crude ash, 17.01–18.72% crude protein, 2.50–2.75% crude fat, 2.46–2.71% crude starch, 4.72–5.19% invert sugar and 1.91–2.10% reduced sugar (Motomura *et al*., 1962). The exine (the outer layer of the membranes of pollen) of *Pinus* pollen is composed of sporopollenin of C₉₀H₁₅₈O₄₄ with hemicellulose (10%) and cellulose (15%), and the intinite (the inner layer) consists of polysaccharides (callose) with pectin (Shaw and Yeadon, 1966; Stanley and Lichtenberg, 1974).

![Fig. 1. Changes in weight loss as a function of temperature for heated pollen grains.](image)

### Table 1. Weight loss, size, elemental compositions and brightness of heated pollen grains

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Weight loss (%)</th>
<th>Size (μm) 95% confidence</th>
<th>Elemental analysis</th>
<th>Brightness (stTAl) 95% confidence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(μm)</td>
<td>Weight (%)</td>
<td>Atomic ratio</td>
</tr>
<tr>
<td>Unheated</td>
<td>0.0</td>
<td>54 ± 1.0</td>
<td>7.80 ± 0.17</td>
<td>52.76 ± 0.29</td>
</tr>
<tr>
<td>66</td>
<td>17.0</td>
<td>49 ± 1.0</td>
<td>7.83 ± 0.17</td>
<td>52.76 ± 0.30</td>
</tr>
<tr>
<td>111</td>
<td>22.3</td>
<td>58 ± 1.5</td>
<td>7.74 ± 0.17</td>
<td>54.33 ± 0.31</td>
</tr>
<tr>
<td>136</td>
<td>22.8</td>
<td>na</td>
<td>7.66 ± 0.17</td>
<td>54.60 ± 0.32</td>
</tr>
<tr>
<td>159</td>
<td>28.0</td>
<td>46 ± 0.9</td>
<td>7.34 ± 0.17</td>
<td>56.01 ± 0.32</td>
</tr>
<tr>
<td>183</td>
<td>39.0</td>
<td>51 ± 0.9</td>
<td>6.32 ± 0.17</td>
<td>57.87 ± 0.37</td>
</tr>
<tr>
<td>194</td>
<td>44.7</td>
<td>45 ± 0.8</td>
<td>5.70 ± 0.17</td>
<td>58.68 ± 0.39</td>
</tr>
<tr>
<td>198</td>
<td>48.8</td>
<td>na</td>
<td>5.40 ± 0.17</td>
<td>58.84 ± 0.42</td>
</tr>
<tr>
<td>203</td>
<td>54.0</td>
<td>47 ± 0.8</td>
<td>4.93 ± 0.17</td>
<td>58.68 ± 0.44</td>
</tr>
<tr>
<td>208</td>
<td>57.8</td>
<td>43 ± 0.7</td>
<td>4.46 ± 0.17</td>
<td>57.33 ± 0.46</td>
</tr>
<tr>
<td>217</td>
<td>64.1</td>
<td>37 ± 0.6</td>
<td>3.66 ± 0.17</td>
<td>56.65 ± 0.48</td>
</tr>
<tr>
<td>220</td>
<td>66.8</td>
<td>na</td>
<td>3.38 ± 0.17</td>
<td>56.55 ± 0.51</td>
</tr>
<tr>
<td>231</td>
<td>69.8</td>
<td>33 ± 0.5</td>
<td>2.84 ± 0.17</td>
<td>56.55 ± 0.61</td>
</tr>
<tr>
<td>251</td>
<td>77.3</td>
<td>na</td>
<td>2.36 ± 0.17</td>
<td>53.63 ± 0.69</td>
</tr>
<tr>
<td>273</td>
<td>82.1</td>
<td>27 ± 0.5</td>
<td>2.09 ± 0.17</td>
<td>54.14 ± 0.78</td>
</tr>
<tr>
<td>290</td>
<td>90.7</td>
<td>24 ± 0.5</td>
<td>2.17 ± 0.17</td>
<td>48.59 ± 0.76</td>
</tr>
</tbody>
</table>

*na*: not analyzed.
Heating experiments on *Pinus* pollen

Fig. 2. Histograms showing size of heated pollen grains. An arrow indicates average of size in each histogram.
SAMPLES AND EXPERIMENTS

Samples
Pollen grains of *Pinus thunbergii* Parl were collected from some pine trees growing on the campus of Hirosaki University (N40°35′12″, E140°28′30″) on May 22nd, 2000.

Heating and weight loss measurements
After being dried overnight in a vacuum desiccator, about 12 mg of pollen grains was put into an aluminum pan and heated in a Rigaku TG8120 thermogravimetric and differential thermal analyzer driven with a Thermo Plus 2 software. The heating time period was compared between 24 and 72 hours under the same conditions other than the period. The noted weight loss occurred continuously on heating from 24 to 72 hours, so 24 hours was adopted as the heating time period. The samples were heated at a fixed temperature between 66 and 290°C for a constant time period of 24 hours in the atmosphere (Table 1).

Before and after heating, the weight of pollen grains in the pan was measured with the differential thermal analyzer.

Size and brightness measurements
A part of the heated powder sample was mounted on a glass slide with “Entellan neu” polymer (Merck Company). The size of bisaccate pollen, the distance between outer edges of both saccuses of a pollen grain in this study, was measured under an Olympus BHS-323 microscope at ×400.

The brightness of saccuses of *Pinus* pollen was measured as follows. Using a transmitted-light microscope with a 100 W halogen bulb at a color temperature of 3000–3100 K, an image of an individual slide-mounted pollen-grain was obtained with Ikegami IF-8500 camera and displayed on a TV monitor. This image was transferred to a Nippon Avionics TV IP-4100 image processor, and then converted to digital data by Ratoc System Engineering Image Command 4198 software. This system can distinguish 256 stages of brightness as grey scale. One measurement of a pollen grain provides a normal distribution in brightness, and then the minimum and the maximum value, the mode and its frequency, and the sum total are recorded. The mean value of the modes in brightness of 100 pollen grains in a sample has been defined as “statistical Thermal Alteration Index” (stTAI) by Ujiié (2001). The methodological details of stTAI measurement were described in Ujiié (2001).

Elemental analysis
After being kept in a vacuum desiccator for a time period longer than overnight, the heated pollen grains were analyzed for carbon, hydrogen and nitrogen content with a Yanaco MT-3 CHN analyzer. The elemental analyses were done according to the standard procedure for the analyzer (Yanaco, 1983).

RESULTS

Figure 1 indicates that weight loss of heated pollen samples increases with increasing temperature. The weight loss rate over 159°C was steeper than that under 159°C. The sample heated at 203°C lost 54% of the initial weight, and at the maximum temperature, 290°C, about 90% was lost (Table 1).

Figure 2 shows the change in size of pollen

---

![Fig. 3. Changes in size as a function of temperature for heated pollen grains.](image-url)
grains, the distance between outer edges of both saccuses of a pollen grain heated at various temperatures. These histograms are grouped into classes by 2 µm in distance. The distance of unheated pollen grains had a range of about 30 µm; a minimum of 43 to a maximum of 70 µm. Although the average value of a hundred pollen grains at each heating temperature lower than 159°C was not settled but scattered between 46 and 58 µm, it had a decreasing trend with increasing heating temperature over 203°C (Figs. 3 and 4).

![Fig. 4. Pollen grains heated at various temperatures.](image-url)
Microfissures on the surface of pollen grains were observed in the samples heated at higher temperature than 203°C under a microscope (Fig. 4).

The contents of carbon, hydrogen and nitrogen in heated and unheated pollen grains are shown in Table 1. The atomic H/C ratio decreased remarkably with increasing heating temperature from 136 to 231°C (Fig. 5). In the range of temperatures lower than 136°C, the decreasing trend of atomic H/C ratio was very gentle and in the range of temperatures higher than 251°C, the ratio was settled at around 0.5.

The atomic N/C ratio of heated pollen grains was almost unchanged at about 0.05 at temperatures lower than 159°C, but abruptly increased at over 183°C (Fig. 6).

Figure 7 shows the results of brightness measurements of pollen grains heated at various temperatures. These histograms are classified into five stages of brightness. The histogram of brightness of unheated pollen grains had a broad distribution across 34 stages. The width of the histograms increased from 60 to 100 stages with increasing heating to temperatures between 183 and 208°C but decreased from 45 to 19 stages at over 217°C. As the lowest limitation of brightness noticed by stTAI measuring system was 43rd stage (Ujiïé, 2001), the value of stTAI at the temperatures higher than 231°C was just a little higher than the recognizable lowest limitation (Table 1).

### DISCUSSION

The heating experiments on living spores of *Lycopodium clavatum* by Yule et al. (1999) indicated a similar trend as seen in the diagram of atomic H/C ratio vs. heating temperature (Fig. 5), although the heating time period of Yule et al. (1999), 60 hours, was two and half times longer than that of this study. In their spore heating experiments, Yule et al. (1999) divided the artificial maturation processes into three stages: the early stage (heating temperature: 25~150°C), characterized by a progressive reduction in atomic H/C ratio; the mature stage (206~212°C), characterized by a rapid decline in H/C ratio; and the post-mature stage (225~300°C), characterized by no decreases but nearly fixed H/C ratios. Yule et al. (2000) showed with the FT-IR spectra increases in the relative intensities of CH\(_2\), CH\(_3\) and =CO bands occurred while no change in the bands due to C=C bonds was noted during the early stages of maturation. During the mature stage the aliphatic content suddenly dropped, coinciding with increases in C=C and =C=O groups. During post-mature stage the aliphatic bands were difficult to resolve by 250°C and the aromatic C=C bands increased with increasing temperature (Yule et al., 2000). The rapid reduction in the atomic H/C ratio of matured spores was indicative of the generation of hydrocarbons from the break down...
Fig. 7. Histograms showing brightness of heated pollen grains. An arrow indicates average of brightness, stTAI in each histogram.
of the spore wall (Brooks, 1980; Marshall, 1991; Yule et al., 1998). Yule et al. (1999) indicated that the rapid decrease in the atomic H/C ratio of artificially heated spores, from 1.46 at 200°C to 0.78 at 212°C, should reflect hydrocarbon generation. In this range of temperature, between 200 and 212°C, the spore transmittance value (% St) proposed as an indicator for organic maturation by Yule et al. (1999), also abruptly increased from 5.5 to 8%. The % St had a correlation with the vitrinite reflectance and an inverse relation with the atomic H/C ratio (Yule et al., 1999).

Sporopollenin of Lycopodium spores and Pinus pollen were similar, and consisted of approximately 10–15% cellulose, 10% xylan fraction and 55–65% lipid fraction (Shaw and Yeaden, 1966). Therefore the assumption for hydrocarbon generation from Lycopodium spores by Yule et al. (1999) may be applicable to the heating experiments on Pinus pollen. The rapid decrease in the atomic H/C ratio, from 1.68 at 136°C to 0.60 at 231°C in Fig. 5 should indicate the hydrocarbon generation by the break down of pollen wall, the degradation of sporopollenin.

The weight loss of heated pollen grains in the temperature range between 136 and 231°C was inferred as the stage of hydrocarbon generation based on the atomic H/C ratio (Fig. 1). However, the size of heated pollen grain, the distance between outer edges of both saccuses of a pollen grain, does not begin to decrease at 136°C, but remains scattered up to 203°C (Fig. 3). It dropped down from the middle point of the stage of hydrocarbon generation. As saccuses of Pinus pollen are mainly composed of gaseous material (Iwanami, 1980), expansion of the gas and deflation caused by degassing through microfissures might take place simultaneously in pollen grains at the low heating temperature. This may be a reason why the size of pollen grains had no settled trend with increasing heating temperature under 194°C. Microfissures on the surface of pollen grains could be observed in the samples at higher temperature than 208°C under a microscope (Fig. 4). At the high heating temperature many microfissures developed on the surface of almost all heated pollen grains so degassing should occur actively and expansion of the gas in pollen should not reflect on the size of pollen grains. Therefore the size decreased with increasing temperature in the high temperature range. Sengupta (1974) reported the similar phenomenon in which minute holes or perforations appeared on the surface of the heated spore grains of Lycopodium clavatum and the inner contents exuded from spore grains through these holes just above 200°C.
The value of stTAI regularly decreased with organic maturation; that of pollen grains from living *Pinus thunbergii* was about 205 (Ujiié, 1996) and that from Recent surface sediment in the Sea of Japan was about 189 (Ujiié, 1998). The threshold of intense oil generation was 110–145 as stTAI in petroleum source rocks (Ujiié, 2001). If this value of stTAI in natural condition can be applied to our heating experiments, the temperature of 203°C should be considered the threshold of intense oil generation (Fig. 8 and Table 1). This temperature is just about the middle point of the stage of hydrocarbon generation indicated by the atomic H/C ratio.

Figure 9 shows the relationship between the atomic H/C ratio and stTAI of heated pollen grains. From the view point of the atomic H/C ratio, the range from 1.68 at 136°C to 0.60 at 231°C might be the hydrocarbon generation stage as stated before. The value of stTAI of pollen grains indicates that intense oil generation might begin at 203°C. The difference between the temperature suggested by the atomic H/C ratio and that by stTAI at the threshold of intense oil generation may be caused by the heating time. Although the former is based on the heating experiments (24 h) in laboratory, the latter is founded on diagenesis and catagenesis (>4.3 million years) in nature.

The type II kerogen, which may be partly derived from pollen and spore materials, generates oil in the range of the atomic H/C ratio from about 1.3 to 0.6 during organic maturation in sedimentary rocks (figure II.7.2 in Tissot and Welte, 1984). The threshold value, 1.68, of the atomic H/C ratio estimated by our laboratory heating experiments is 0.38% higher than that estimated for natural hydrocarbon generation from type II kerogen. This difference should be due to the duration of heating. The atomic H/C ratio, 1.01, at the threshold (203°C) estimated by stTAI is 0.29% lower than that estimated for natural hydrocarbon generation. Therefore these heating experiments indicated that hydrocarbon generation from living-pollen grains should take place in the temperature range between about 135 and 230°C based on the atomic H/C ratio (Fig. 5).

During late diagenesis to metagenesis large quantities of carbon dioxide, water, heteroatomic (nitrogen, sulfur and oxygen) compounds and hydrocarbon were produced from organic matter so the relative concentration of carbon in sedimentary organic matter gradually increased (Tissot and Welte, 1984). Therefore, it has been generally believed that the atomic N/C ratio of organic matter in sediments gradually decreases with maturation. But this study showed that the atomic N/C ratio of heated pollen grains settled at around 0.05 at temperatures lower than 159°C, and abruptly increased at temperatures over 183°C (Fig. 6). These phenomena in heated pollen or spores have not yet been reported. Experimental evolution of various coals seemed to confirm the view that the nitrogen present in organic matter was affected little until an advanced stage of thermal evolution was reached and the activation energy of the N₂ generation ranged from 40 to 70 kcal mole⁻¹ (Tissot and Welte, 1984). This activation energy for N₂ generation from coals was nearly equal to or higher than 50 kcal mole⁻¹ of that for oil generation from type II kerogen (Tissot and Welte, 1984). The change in the atomic N/C ratio in Fig. 6 is concordant with the results of the experimental evolution of coals. The details of mechanism of increasing N/C ratio in heated pollen-grains must be solved by further investigation.

**Conclusions**

Heating experiments on living-pollen grains of *Pinus thunbergii* showed decreasing trends of weight, size, atomic H/C ratio and stTAI with increasing temperature. However, the atomic N/C ratio indicated an increasing trend in the range of high heating temperature.

With the comparison with the heating experiments of spores by Yule *et al.* (1999), it was inferred that intense oil generation from pollen grains should take place in the temperature range of about 135 and 230°C based on the rapid decrease of the atomic H/C ratio. The changes in brightness, stTAI, of heated pollen-grains indicate that oil generation might begin at about 200°C.
based on the application of the data in natural organic maturation in sedimentary rocks to these experiments. The difference between the temperature suggested by the atomic H/C ratio and that by stTAI at the threshold of intense oil generation may be caused by heating time. Therefore hydrocarbon generation from living-pollen grains should take place in the temperature range between about 135 and 230°C, based on the atomic H/C ratio.

Acknowledgments—We thank Dr. Shiba, M. for his assistance in operating a Rigaku TG8120 thermogravimetric and differential thermal analyzer. Two anonymous referees are thanked for constructive and useful reviews.

REFERENCES


