A trial of density pollen fractionation from sediment core samples for the purpose of pollen AMS dating: evaluation and perspectives

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1. Introduction

Radiocarbon dating has been widely used in the field of Quaternary science as an efficient means of age measurement. Especially, since its invention, the method of AMS (accelerator mass spectrometry) dating has demonstrated a particular value for its remarkably small requirement of the sample quantity (order of milligram-size carbon) in comparison with other conventional methods of radiocarbon dating (for example gas spectrometry and liquid scintillation counter, which require the sample volume in the order of gram carbon). One of the important advantage of this reduced sample requirement is that it enables us to severely choose the dating samples. For example, in the case of Quaternary science, it was not until the introduction of AMS dating that it became possible to date only one piece of terrestrial macro-remains like a conifer needle or an arboreal seed. Such a precision of sample is very needed because otherwise really good age control of the sediment core can not be expected. Consequently, more and more terrestrial macro-remains have nowadays been dated in the Quaternary science, revealing the more details of the Quaternary environmental changes with higher resolution of the age determination (for example, Ammann & Lotter, 1989 ; Brugiapaglia, 1996)

However, as is for example typically the case with European late-Glacial lacustrine clay sediment, the terrestrial macro-remains are not always available from all the expected horizons. In such cases, the limiting factor of the radiocarbon dating is no longer the performance nor the resolution of the dating equipment, but the nature of the sediment it self.

Pollen extraction and purification from the sediments can be a solution for this problem, the fossil pollen grains being the most probably terrestrial and contemporary with the sediment matrix. Very recently, some

techniques of the density pollen fractionation were proposed by Forster & Flenley (1993), Regnéll & Everitt (1996) and Nakagawa *et al.* (in press). Given these new technical proposals, during the routine palynological studies of our laboratory (Laboratoire de Botanique Historique et Palynologie, Université d'Aix-Marseille III, France), we carried out a series of dating trial on pollen concentrates as well as on the terrestrial macro-remains. This report presents the results of these trials, and discusses the present reliability of the pollen concentration method for the purpose of AMS dating.

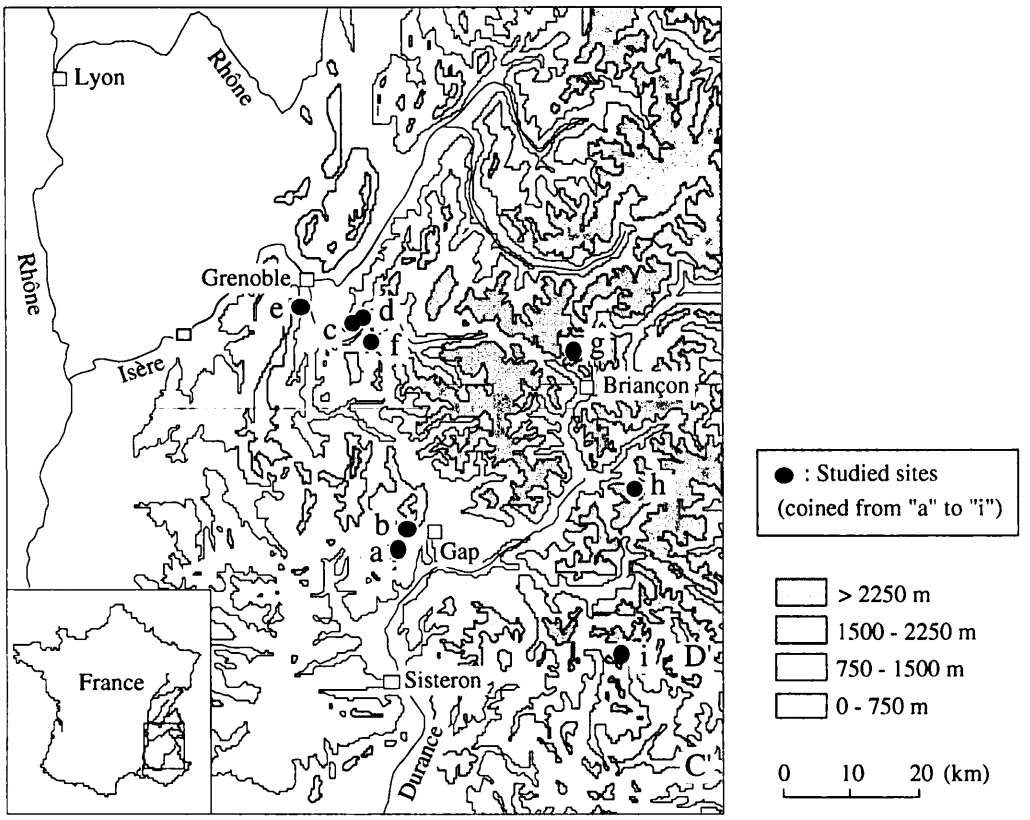

Fig. 1 : Nine studied sites distributed in the French southern to central Alps. a: Raux peat bog, b: Correo peat bog, c: Mont Sec peat bog, d: lake Praver, e: Peuil peat bog, f: lake Boites, g: lake Cristol, h: lake Miroir, i: lake Lignin.

2. Materials and methods

Core samples are obtained from nine lakes and peat bogs distributed in the southern to central French Alps (Fig. 1). Fossil pollen concentrates and terrestrial macro-remains are extracted from the totals of 7 and 33 selected horizons, respectively, and AMS ^{14}C dated. The pollen concentrates were prepared by the method modified after Regnéll & Everitt

(1996). The terrestrial macro-remains were visually picked up from the section of the core samples, or extracted by sieving with running water. About 5 grams of sediment for each horizon was introduced into the pollen extraction, whose processing sequence was as follows:

1) HCl treatment (36 % HCl at room temp., 12 hours)
2) wash with water
3) NaOH treatment (10 % at 90 °C, 10 minutes)
4) repeated rinsing and centrifugation with water (for 6 times)
5) HCl treatment (36 % HCl at room temp., few minutes)
6) density separation, preparative centrifugation (repeatedly at many different densities - See below.)

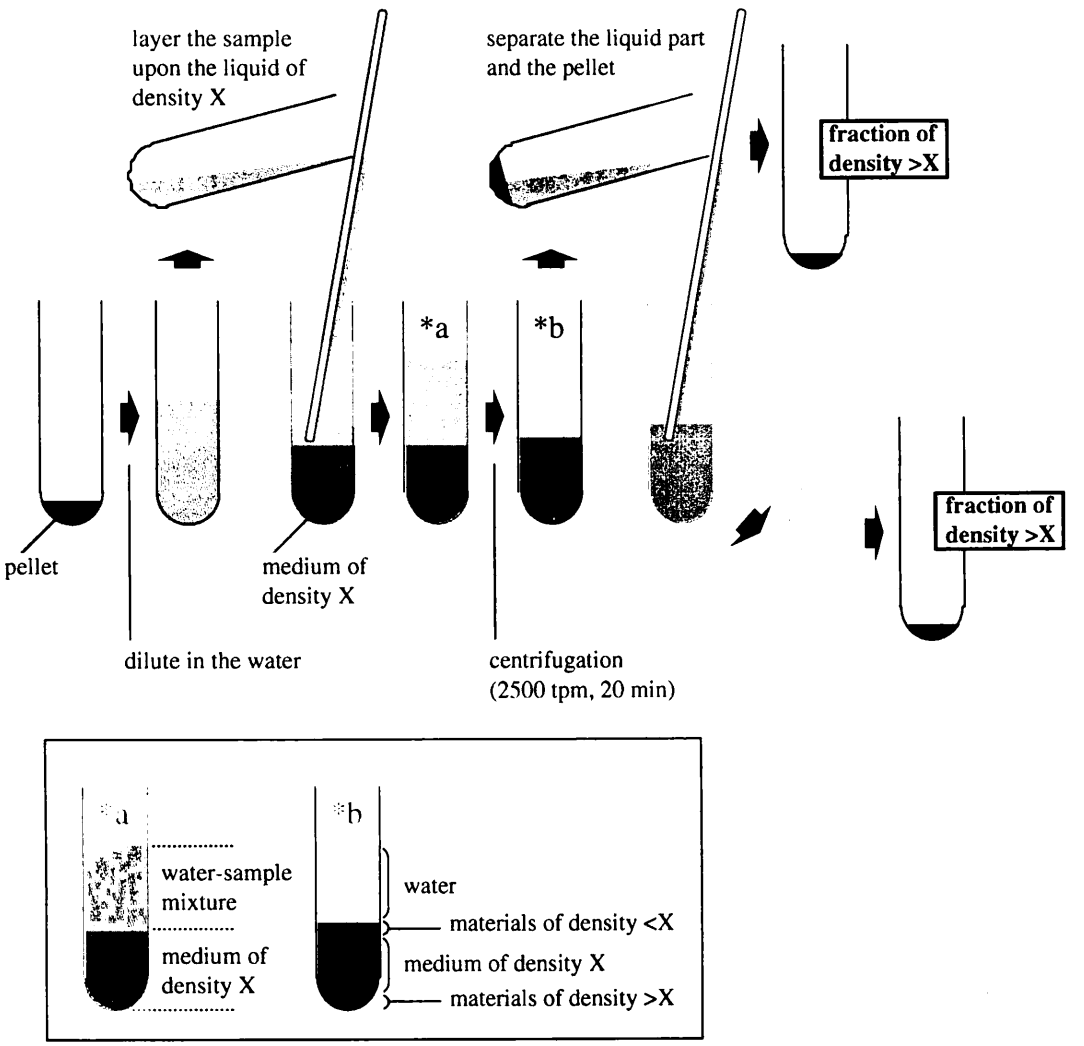

Fig. 2 : Procedure to separate different density fractions of the sediment (modified after Regnéll & Everitt, 1996).

The step 6) is described in more detail in the figure 2. The sample is mixed with water and carefully layered on the dense medium liquid. A solution of KI and CdI_2 (10 to 11 in w/w proportion) was used as the dense medium for the reason of its lower viscosity. Then the centrifugation was performed at 1,800 rpm for 20 minutes. The lighter material is transferred into another tube, diluted in water, and recovered by centrifugation at 2200 rpm for 3 minutes. This treatment was repeated at least twice at the same density (both for lighter and heavier materials) in order to achieve better density fractionation. The fractions were then washed twice by water, treated by HCl, and introduced into another turn of density separation at another density. For each turn, a slightly lighter density than previous times was selected ; *i.e.*, we began from density 2.00, then descended to 1.95, 1.90, 1.85 ..... finally down to 1.20. The density fractionation was repeated at least twice for each density. Each density fraction was observed by light microscopy, and only the richest fraction(s) in pollen were submitted to AMS ^{14}C dating. However, not all the submitted samples were really pure in pollen. Both pollen and macro-remains samples were transformed into graphite powder by the method of Vogel *et al.* (1987) and Kitagawa *et al.* (1995), and AMS ^{14}C dated by the corroboration of Nagoya University, Japan and Groningen University, the Netherlands.

3. Results and discussion

3.1. General condition of the pollen concentrates and dating results

The results of extraction and dating of 40 samples shows in Appendix. Most of the density fractions were not really pure in pollen. More in details, the Holocene samples tended to be contaminated by organic micro fragments (peat fragments, planktonic dead bodies, etc.) and the two late-Glacial samples (sample No. 11 and 12) were rather rich in carbon particles than in pollen. The age values ranged between 27650 ±150 BP (sample No. 12) and 450 ±50 BP (sample No. 13), showing that the samples are mainly from late-Glacial to Holocene as had previously been expected.

3.2. Evaluation of the dating results on pollen concentrates

The sampling depth, the dates of density fractions, and the dates of macro-remains are compiled in the figure 3. The dates of the density fraction

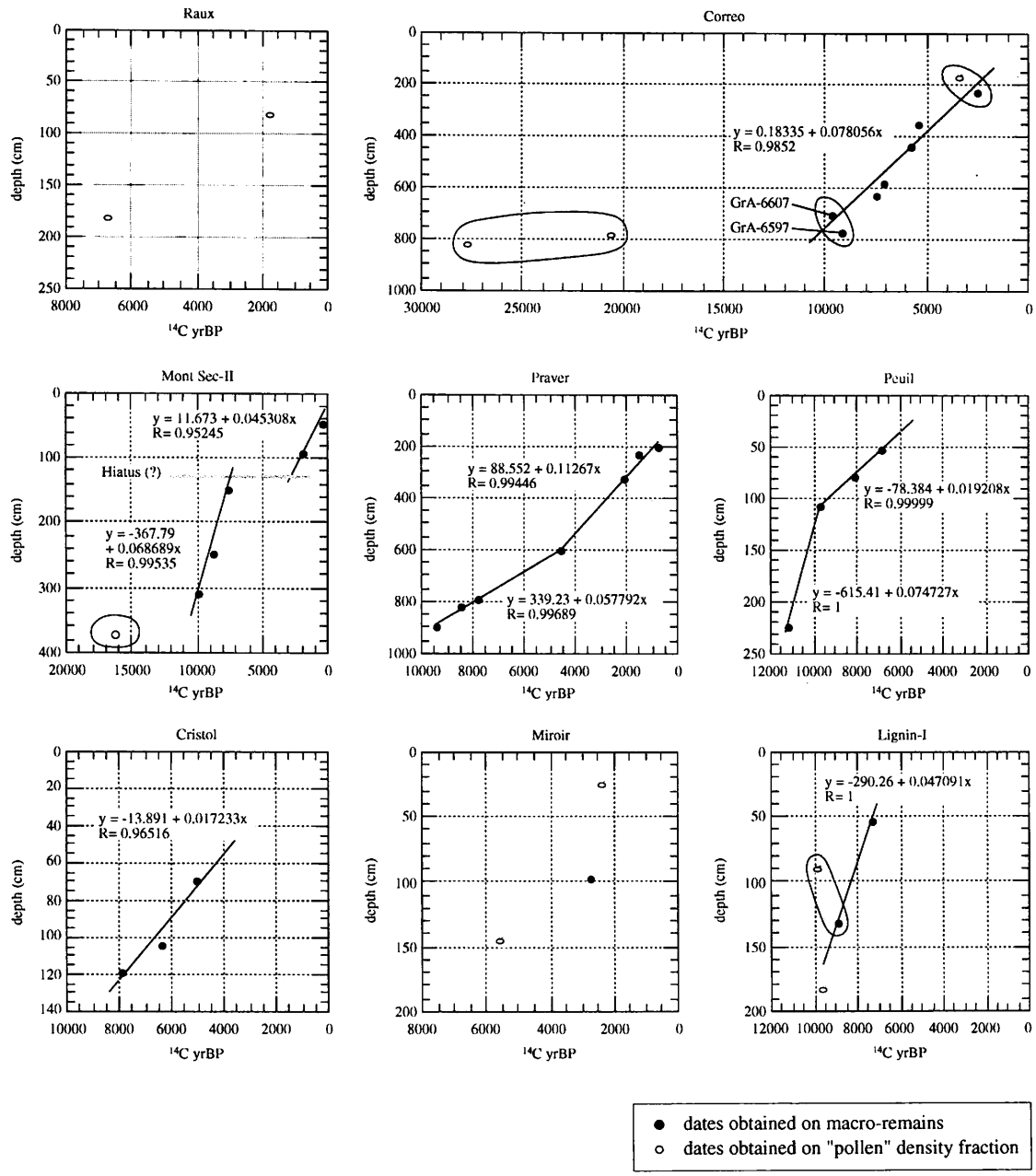

Fig. 3 : Depth - age distribution of the dating results. The dates of "pollen" concentrates tend to shft older than those of terrestrial macro-remains.

and macro-remains are given in open and closed dotes, respectively. Such presentation serves to detect the anomalies (inversion and jump) of age-depth distribution. It is clearly shown that the dates of macro-remains are generally aligned on the reasonable tendencies (with only one exception of samples No. 4 and 5 in Correo peat bog which are inverted in age),whereas

the dates of pollen concentrates (density fractions) almost always show anomalies: with only one exception of the sample No. 40, all the dates of density fractions are considerably older than those of terrestrial macro-remains. It thus seems that the density fraction prepared by the method of Regnéll & Everitt (1996) are still contaminated by the materials that contain the carbon derived from older time. In the cases of late-Glacial clay samples, it is undoubtedly the carbon particles that cause the age shifts. They can even be the particles that had been long conserved in the Glacial ice for the preceding dozens of millennia. In the cases of Holocene organic samples, on the other hand, the main source of the "older" carbon is probably aquatic vegetal fragments that can easily be affected by hard water effect.

3.3. Some possible solutions for the imperfection of the method

Nakagawa *et al.* (in press) developed the dense-media separation method of pollen enrichment for use with organic sediment samples. According to their arguments, the best density to separate pollen grains from the organic sediment matrix lies around 1.88 g/cm^3, 1.90 being too heavy and 1.85 being to light. So the required precision for the density control of the liquid medium is less than 0.5 g/cm^3, which was the resolution of the density fractionation adopted in this report. The increase of the density resolution might enable to achieve better pollen separation from the organic sediments. As for clay samples, Certain limit exists for the density separation of pollen grains from carbon particles, the density of which overlaps with that of fossil pollen grains (Carcaillet, personal communication). Chemical procedures to decompose carbon particles are therefore needed to obtain highly concentrated pollen pellet from the late-Glacial clay sediments. The use of Schulze liquid, a liquid long used to decompose coals, might be a solution for this problem. We will take an action to verify this possibility in very near future.

The sample No. 40 was only one pollen concentrate the date of which gave a good accordance with the tendency given by the dates of macro-remains. This sample was nearly perfectly pure in pollen, as was in fact scarcely the case with the most of other density fraction samples. This implies that the potential value of pollen dating is still considerably high, requiring only a good routine method of its extraction in enough quantity.

4. Remarks

Given these rather unexpected results, we contacted personally to Drs. Regnéll and Everitt (the authors of the paper on the extraction methodology) in order to ask the performance of the method in their laboratory. The answer was that their method works generally well for poorly humified peat samples, but scarcely for gyttja or clay samples, as was well in accordance with our own results. In conclusion, we probably have to accept the idea that no pollen purification method has been developed to as satisfactory degree as we can adopt in the laboratory routine works, as yet. The establishment of the stand up procedure of the pollen concentration and purification method suitable for AMS dating remains as a future perspective. The results of some trials suggested in this report will be soon reported when they will get available.

Acknowledgements

We are grateful to Dr. T. Nakamura of Nagoya University, Japan and Dr. J. van der Plicht of Groningen University, the Netherlands for their invaluable corporation, Dr. J. -L. de Beaulieu of Université d'Aix-Marseille III,France for his useful suggestions and encouragement.

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Appendix: The results of all the datings _

site	depth (cm)	No.	materials (condition)	radiocarbon age	Code
Raux	64 - 66,5 1		density fraction 1	1770 ±100 yrBP	NUTA
Raux	163 - 168,5	2	density fraction 1	6680 ±100 yrBP	NUTA
Correo	171 - 174	3	density fraction 2	3340 ±80 yrBP	GrA-6614
Correo	227,5	4	a piece of wood of Betula sp.	2580 ±80 yrBP	GrA-6601
Correo	351 - 352,5	5	unidentified leaf fragments	5450 ±80 yrBP	GrA-7789
Correo	440	6	unidentified leaf fragments	5810 ±80 yrBP	GrA-6612
Correo	580,5 - 584,5	7	needles of Abies sp.	7110 ±80 yrBP	GrA-6602
Correo	629 - 632	8	a needle of Abies sp.	7550 ±80 yrBP	GrA-6595
Correo	699 - 702	9	seeds of Betula sp.	9670 ±90 yrBP	GrA-6607
Correo	763,5 - 770,5	10	seeds of Betula sp.	9220 ±480 yrBP	GrA-6597
Correo	778 - 784	11	density fraction 2(rich in carbon)	20620 ±120 yrBP	GrA-6583
Correo	822 - 825	12	density fraction 2(rich in carbon)	27650 ±150 yrBP	GrA-6589
Mont Sec	45,5 - 49,5	13	a wood fragment of Fagus sp.	450 ±50 yrBP	GrA-7790
Mont Sec	90,5 - 92,5	14	a wood fragment of Betula sp.	1850 ± 60yrBP	GrA-7791
Mont Sec	145,5 - 149,5	15	an unidentified wood fragment	7560 ±90 yrBP	GrA-6599
Mont Sec	246 - 249	16	a wood fragment of Betula sp.	8830 ±100 yrBP	GrA-7792
Mont Sec	310	17	a wood fragment of Tilia sp.	9940 ±140 yrBP	GrA-6600
Mont Sec	370 - 374,5	18	density fraction 3	16110 ±100 yrBP	GrA-6605
Praver	197,5	19	an unidentified leaf	810 ±80 yrBP	GrA-6580
Praver	231 - 234	20	needles of Abies sp.	1520 ±120 yrBP	NUTA
Praver	327	21	an unidentified leaf	2060 ±80 yrBP	GrA-6582
Praver	603	22	needles of Abies sp.	4540 ±80 yrBP	GrA-6586
Praver	795	23	needles of Abies sp.	7830 ±320 yrBP	GrA-6608
Praver	815,5 - 819,5	24	needles of Abies sp.	8520 ±90 yrBP	GrA-6588
Praver	893 - 897	25	an unidentified wood fragment	9460 ±90 yrBP	GrA-6587
Peuil	50,5 - 54,5	26	a wood fragment of Betula sp.	6810 ±80 yrBP	GrA-6585
Peuil	75,5 - 79,5	27	a wood fragment of	8120 ±90 yrBP	GrA-6590
		28	Pinus sylvestris / uncinata		
Peuil	105,5 - 109,5	29	unidentified wood fragments	9670 ±90 yrBP	GrA-6610
Peuil	224	30	a wood fragment of Betula sp.	11230 ±90 yrBP	GrA-6584
Cristol	67,5 - 70	31	a needle of P. cembra	5040 ±80 yrBP	GrA-6611
Cristol	102,5 - 105	32	needles of Larix sp.	6380 ±90 yrBP	GrA-6613
Cristol	117,5 - 120	33	wood fragments of Betula sp.	7910 ±80 yrBP	GrA-6609
Miroir	23 - 27	34	density fraction 2	2340 ±70 yrBP	NUTA
Miroir	95 - 99	35	a wood fragment of Larix sp.	2730 ±80 yrBP	NUTA
Miroir	143,5 - 145,5	36	density fraction 1	5580 ±90 yrBP	NUTA
Lignin	52 - 55	37	leaf fragments of Betula sp.	7300 ±80 yrBP	NUTA
Lignin	89 - 91	38	density fraction 2	9790 ±90 yrBP	GrA-6616
Lignin	132	39	a wood fragment of Sarix sp.	8970 ±90 yrBP	GrA-6615
Lignin	180 - 185	40	density fraction 2(pure in pollen)	9560 ±160 yrB	NUTA

density fraction 1 : 1.25 - 1.55 g/ g/cm^3, richest in pollen, density fraction 2 : 1.25 - 1.60 g/cm^3, 3, richest in pollen density fraction 3 : 1.55 - 1.60 g/cm^3, richest in pollen

ＡＭＳ年代測定を目的として、堆積物コアサンプルから花粉のフラクションを分離する試みについて：技術の評価と展望

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フランス南部および中部アルプス地方の９地点から堆積物試料を採取し、層準によって化石花粉に富んだ密度フラクションまたは陸上起源の大型植物遺骸を抽出し、それぞれに対してＡＭＳ年代測定をおこなった。花粉のフラクションの分離にあたっては、Regnéll & Everitt (1996) によって提案された比重分離法を適用した。もっとも彼らの論文が示唆するところとは異なり、実際に抽出されたフラクションは、かならずしも花粉だけを純粋に含むものではなかった。年代測定の結果を比較・検討したところ、密度フラクションから得られる年代は、陸上起源の大型植物遺骸から得られる年代に対して、ほぼ一貫して古い値をしめすことが分かった。ただし分離されたフラクションがほぼ純粋に化石花粉を含む場合に限っては、両者の間に値の矛盾は見られなかった。このことは、年代のシフトには花粉遺骸の物質の混入が寄与していること、また、花粉が純粋な形で抽出できさえすれば、ＡＭＳ年代測定用の適切な試料となりうることを示している。Regnéll & Everitt の方法は原状では完全なものではなく、このことは、その後の彼らとの personal communication によっても裏付けられている。技術的な改善点としては、密度のさらなる細分化、シュルツ液の利用による炭素粒子の除去などが考えられ、これらの実施は当面の課題であろう。