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Short Article

Opal Phytolith Assemblage and Its Relation with Plant Biomass

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The present methods of opal phytolith analysis still limits an accurate reconstruction of past environment. In this paper opal phytolith assemblage and its relation with plant biomass was studied. Opal phytolith production of some common grass and tree species was determined mixing leaf blades at different ratios. All plants showed some variability in the production of opal phytoliths, especially for those from Bulliform cells (Fan shape). The results showed that leaf blades of *Sasa kurilensis* produce about 23 times more Fan shape phytoliths and 27 times more Bambusoid phytoliths than the quantity of Jigsaw-puzzle opal phytoliths produced by *Fagus crenata* leaves. Generally, to have similar amounts of tree and grass origin opal phytoliths in soils, the quantity of tree leaves should be more than 10 times of that of the grasses. The clear results obtained for the plant species studied show that the determination of opal phytolith ratios between species is a good tool to estimate plant biomass and consequently to achieve a more precise reconstruction of past vegetation.

Key Words : opal phytolith assemblage, opal phytolith differential production, past environment reconstruction, plant biomass

I. Introduction

One of the main problems of opal phytolith research is the quantification approach used in phytolith analysis (Pearsall, 1989 ; Kondo, 1995). Such problems are responsible for the under- and over-representation of plant taxa that produce different quantities of silica bodies and also a limitation to characterize abundance of silica bodies that can not be meaningfully counted. These problems still limit the estimation of past environments. Plants that produce few amounts of opal phytoliths or forms that are also found in other species, may be easily ignored even though they are present as part of the vegetation. Phytolith assemblages have been used to reconstruct past vegetation and climate (Fujiwara, 1976, 1984 ; Kondo *et al.*, 1988 ; Sase *et al.*, 1990, 1995 ; Fredlund and Tieszen, 1994 ; Inoue and Sase, 1996), and its use has reached to the level of plant communities and their relation with soil taxonomy (Kondo and Iwasa, 1981 ; Kawamuro and Osumi, 1988 ; Kondo *et al.*, 1988). However, the differential production between species and the broad size range of phytoliths (less than 5 to 500μm) in the same species make difficult the quantification of vegetation cover through conventional opal phytoliths assemblage. Furthermore, studies on opal phytolith assemblage and its relation with biomass is still scanty (Fujiwara and Sasaki, 1978 ; Kondo, 1983 ; Sugiyama, 1999). In a former work (Takachi *et al.*, 2000), the extremely small amounts of opal phytoliths from *Fagus crenata* in the natural forest soils and

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Zoysia japonica in the grassland soils led the conclusions to be based more on the experience of the authors and local vegetation characteristics than on the opal phytolith data obtained in these areas. This result showed that the determination of opal phytoliths ratios is an important factor to achieve a reliable estimation of past vegetation.

The objective of this work is to optimize the relationship between different classes of opal phytoliths and their production in some common plant species of Japan, in order to clarify opal phytolith assemblages in soils and their relationship with vegetation cover.

II. Materials and methods

Leaf samples from grass species (*Miscanthus sinensis, Zoysia japonica*), dwarf bamboo (*Sasa hastatophylla, Sasa kurilensis* and *Pleioblastus simonii*), and trees (*Fagus crenata, Picea ghlenii, Castanopsis cuspidata,* and *Persea thunbergii*) representing common species of the vegetation in Japan, were collected at their most matured stage (end of September and October). The samples were washed, cut into small pieces, dried overnight at 110℃ and mixed at different ratios according to the estimated opal phytolith content of each species (Table 1). During the weighting of the samples, a special care was taken to take an significant and homogene quantity of at least 0.5g of each leaf sample. Extraction of opal phytoliths were performed according to the method described by Kondo and Sase (1986). The samples were treated with a mixed acid solution (H_2SO_4 : HNO_3 : $HClO_4$ at a ratio of 1 : 10 : 4), to digest organic matter. The samples were subjected to ultrasonication and the 5 to 200μm fractions were separated through decantation and siphoning. A small amount of the separated fraction was mounted on a microscope slide glass with clove oil and opal phytoliths were identified and counted. Five to 7 repetitions of about 500 grains were counted using a polarizing microscope (400× magnification) and opal phytolith ratios were determined.

It is important to emphasize that errors could occur during the preparation of the materials, resulting in misinterpretations. In this study part of the experiment had to be restarted due to problems with the dispersion of the particles. Samples composed of leaves collected from living plants, and consequent absence of degradation process of organic matter (i.e. leaves), required a longer ultrasonic treatment (3 min. at 350 W, 20 Kc/s) to obtain complete dispersion of the opal phytoliths.

III. Results and discussion

Table 1 and Figure 1 indicate that the ratios of *Fagus crenata* (Plate 2, nos. 1 and 2) and *Sasa kurilensis* (Plate 1, nos. 3 and 4) have a relatively uniform and low standard deviation (SD), a uniform normalized values (NV : SD/mean number of phytoliths counted) with the increase of mixing ratios and an extremely unstable quantitative equivalence (e.g. considering that *Fagus* as species A and *Sasa* as species

Table 1 Opal phytolith ratios of selected plant species

Plant species (Place collected)	Leaf-Mixing ratio	(b)	Ratio of the counted numbers of selected opal phytoliths															
			Mean(a)	SD	NV	QE	Mean(a)	SD	NV	QE	Mean(a)	SD	NV	QE	Mean(a)	SD	NV	QE
			Jigsawpuzzle/Fan				Jigsawpuzzle/Bambusoid											
Fagus crenata /	1:1	1	0.052	0.016	0.32	20.0	0.042	0.010	0.25	25.0								
*Sasa kurilensis**	2:1	2	0.17	0.067	0.40	11.8	0.14	0.058	0.42	14.3								
(Towada, Aomori Pref./	4:1	4	0.18	0.061	0.34	22.2	0.22	0.062	0.28	18.2								
Fuji Bamboo Garden,	10:1	10	0.36	0.10	0.27	27.8	0.30	0.16	0.53	33.3								
Shizuoka Pref.)	15:1	15	0.45	0.06	0.14	33.3	0.35	0.15	0.42	42.9								
			Fan/Fan				Fan/Bambusoid				Chloridoid/Fan				Chloridoid/Bambusoid			
Zoysia japonica /	1:1	1	0.23	0.040	0.17	4.35	0.28	0.080	0.29	3.57	7.64	2.17	0.28	0.13	8.71	0.86	0.10	0.11
*Sasa hastatophylla***	2:1	2	0.71	0.10	0.14	2.82	0.84	0.13	0.15	2.38	17.8	3.27	0.18	0.11	20.6	1.99	0.10	0.10
(Kamisodegawa, Iwate Pref./	4:1	4	1.33	0.14	0.11	3.11	1.34	0.21	0.16	2.98	41.1	6.96	0.17	0.10	41.2	5.70	0.14	0.10
Fuji B. Gdn, Shizuoka Pref.)	8:1	8	4.85	1.20	0.25	1.65	2.46	0.94	0.38	3.25	105.1	22.4	0.21	0.08	56.3	7.16	0.13	0.14
			Fan/Fan				Fan/Bambusoid				Panicoid/Fan				Panicoid/Bambusoid			
Miscanthus sinensis /	1:1	1	0.37	0.18	0.49	2.70	0.03	0.025	0.83	33.3	7.60	7.72	1.02	0.13	0.89	0.067	0.08	1.12
*Pleioblastus simonii***	2:1	2	0.77	0.53	0.69	2.60	0.09	0.040	0.44	22.2	8.70	5.64	0.65	0.22	1.30	0.083	0.06	1.54
(Obihiro, Hokkaido Pref./	4:1	4	1.06	0.41	0.39	3.77	0.17	0.032	0.19	23.5	14.0	3.80	0.27	0.29	3.06	0.33	0.11	1.31
Fuji B. Gdn, Shizuoka Pref.)	8:1	8	1.94	0.31	0.16	4.12	0.58	0.25	0.43	13.8	19.4	7.69	0.40	0.41	4.49	0.52	0.12	1.78

SD: standard deviation; NV: normalized value (SD/a); QE: Quantitative equivalence (b/a: estimated number of b when a=1)

* average of 7 repetitions; ** average of 5 repetitions; about 500 phytoliths were counted in each repetition.

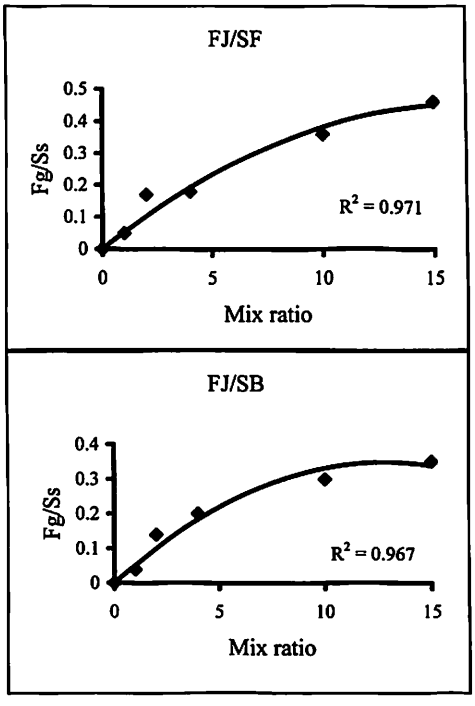

Fig. 1 Variation in the ratios of *Fagus crenata* and *Sasa kurilensis*

Plots in the graph are average of 7 repetitions of about 500 phytoliths counted. FJ : *Fagus* origin jigsaw-puzzle, SF : *Sasa* origin Fan, SB : *Sasa* origin Bambusoid, Fg/Ss : *Fagus crenata* and *Sasa kurilensis* ratios, Mix ratio : quantity of *Fagus* leaves added to 1 of *Sasa*.

B, then QE is the number of phytoliths of species B when the number of phytoliths of species A is one for any mixing ratio) which theoretically, should be the same with the increase of mixing ratio. These results show that the production of opal phytoliths of these plants are relatively uniform throughout their leaf surface. However, the quantitative equivalence of Fan shape (Bulliform cell) and Jigsaw-puzzle shape shows that either one or both species have a variable production of phytoliths in general. In this case, it is probably *Fagus crenata*. *F. crenata* has a far lower opal phytolith production than *Sasa kurilensis*, showing differential production between the two species. Both *F. crenata* and *S. kurilensis* (Jigsaw-puzzle / Fan shape phytoliths and Jigsaw-puzzle / Bambusoid phytoliths) show relative uniformity in their phytolith production. The low standard deviation observed is probably due to the clear difference and distinction between the types of opal phytoliths used to determine the ratios (i.e. Fan shape and Bambusoid classes from *Sasa* and Jigsaw-puzzle shape from *Fagus*). For each Jigsaw-puzzle shape the quantity of Fan shape found ranged from 11.8 to 33.3, while Bambusoid ranged from 14.3 to 42.9. It is probably due to the weak nature of Jigsaw-puzzle shaped opal phytoliths observed due to its uneven surface with numerous small holes, which make them more fragile and consequently easier to break. Through average values, it can be estimated that leaves of *Sasa kurilensis* produce about 23 times more Fan shape phytoliths and 27 times more Bambusoid phytoliths than *Fagus crenata* production of Jigsaw-puzzle opal phytoliths.

Zoysia japonica (Plate 1, nos. 7 and 8) and *Sasa hastatophylla* also show a similar tendency as regards variable quantitative equivalence for Fan shaped opal phytoliths. However, their ratio generally show, lower NV and as mentioned above, except for Fan/Fan, higher stability for quantitative equivalence. For each Fan shape of *Zoysia* origin, Fan shape and Bambusoid classes phytoliths from *Sasa* range from 1.65 to 4.35 and 2.38 to 3.57, respectively. It shows that the production of Fan shape of *Zoysia* origin is also moderately irregular on the leaf. The results show that leaves of *Sasa hastatophylla* produce 3 times more Fan shape and Bambusoid class opal phytoliths than the quantity of Fan shape produced by *Zoysia japonica*. However, when compared to Chloridoid class from *Zoysia*, *Sasa* produces 0.1 times of both Bambusoid and Fan shape classes (Fig. 2).

Miscanthus sinensis (Plate 1, nos. 5 and 6) and *Pleioblastus simonii* (Plate 1, nos. 1 and 2) opal phytolith ratios also show a tendency similar to those discussed above. Although they show a good correlation (Fig. 3), quantitative equivalences of Fan shape opal phytoliths from leaves of *Miscanthus sinensis*, and Fan shape and Bambusoid class from *Pleioblastus simonii* show a broad range. Quantitative equivalence of Fan shape and Bambusoid class from *P. simonii* ranges from 2.60 to 4.12 and 13.8 to

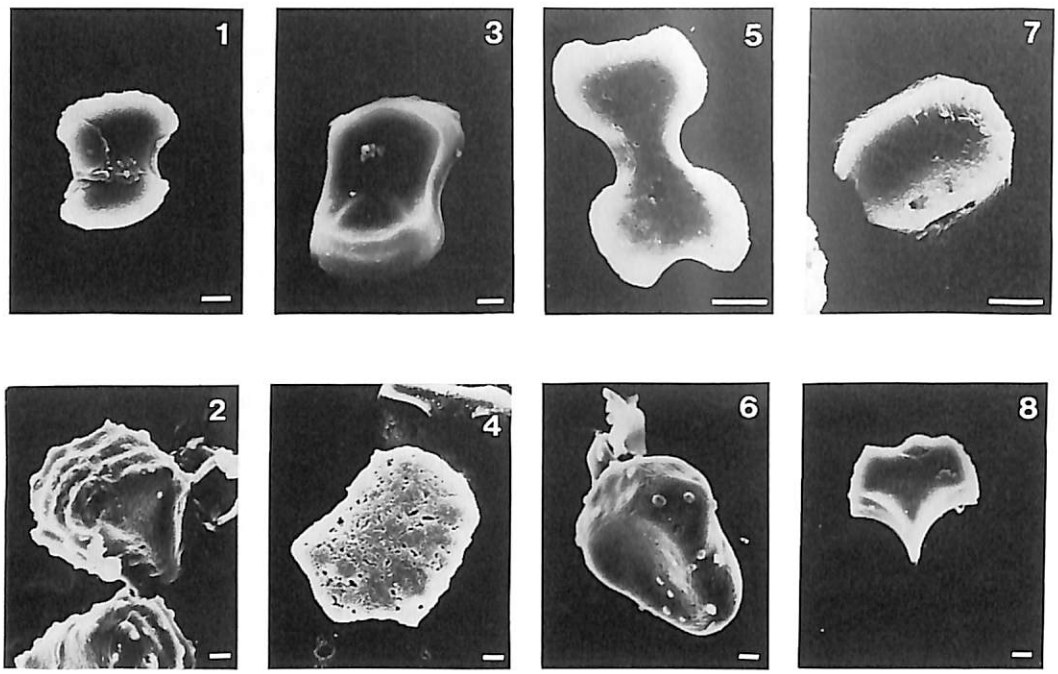

Plate 1 Scanning electron microscope photographs of phytoliths from grasses
Short cell phytoliths : 1, 3, 5, 7. Bulliform cell phytoliths : 2, 4, 6, 8.
Pleioblastus simonii : 1, 2, *Sasa kurilensis* : 3, 4, *Miscanthus sinensis* : 5, 6, *Zoysia japonica* : 7, 8. Scale bar=5μm

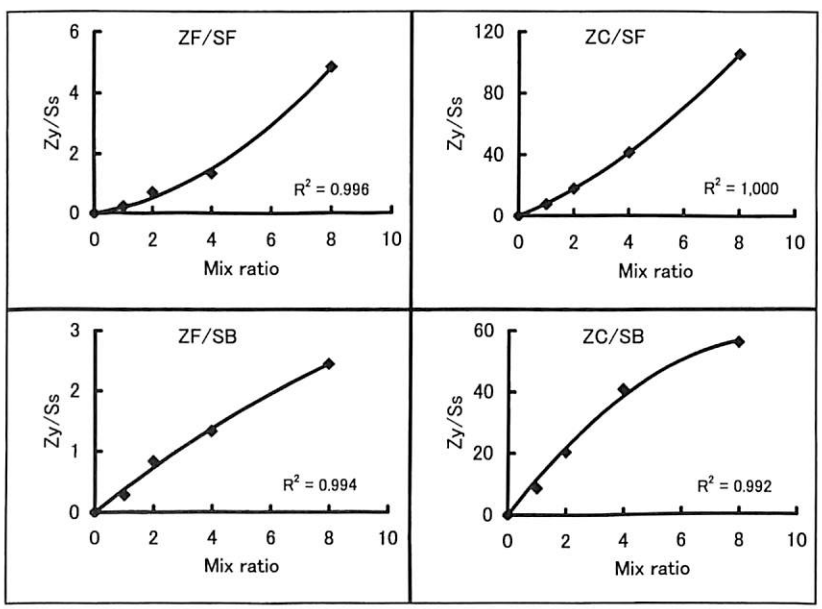

Fig. 2 Variation in the ratios of *Zoysia japonica* and *Sasa hastatophylla*
Plots in the graph are average of 5 repetitions of about 500 phytoliths counted. ZF : *Zoysia* origin Fan, SF : *Sasa* origin Fan, ZC : *Zoysia* origin chloridoid, SB : *Sasa* origin Bambusoid, Zy/Ss : *Zoysia japonica* and *Sasa hastatophylla* ratios, Mix ratio : quantity of *Zoysia* leaves added to 1 of *Sasa*.

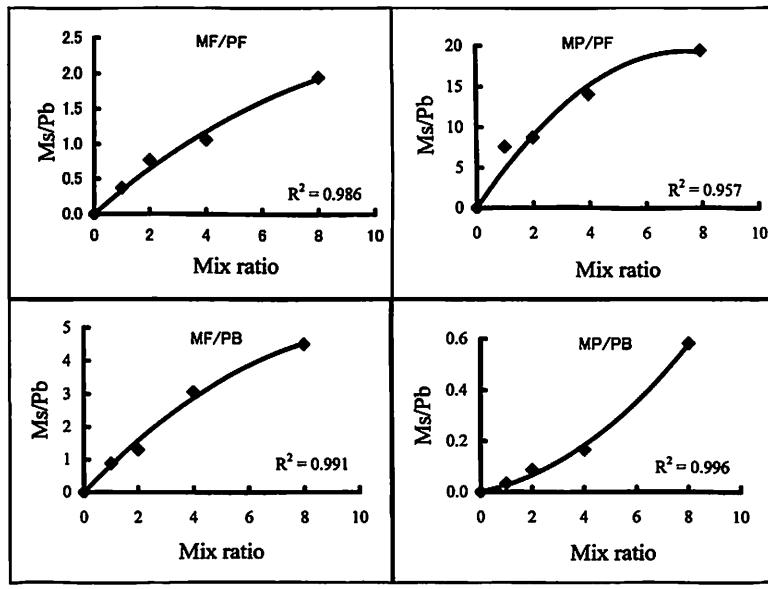

Fig. 3 Variation in the ratios of *Miscanthus sinensis* and *Pleioblastus simonii*
Plots in the graph are average of 5 repetitions of about 500 phytoliths counted. MF : *Miscanthus* origin Fan, PF : *Pleioblastus* origin Fan, MP : *Miscanthus* origin Panicoid, PB : *Pleioblastus* origin Bambusoid, Ms/Pb : *Miscanthus sinensis* and *Pleioblastus simonii* ratios, Mix ratio : quantity of *Miscanthus* leaves added to 1 of *Pleioblastus*

33.3, respectively, in relation to Fan shape of *Miscanthus* origin. Furthermore, the quantitative equivalence of Panicoid class from *Miscanthus* to Fan shape phytoliths from *Pleioblastus* also show a wide variation (0.13 to 0.41), while that of Panicoid to Bambusoid class show a narrower one (1.12 to 1.78). *Pleioblastus* leaves produce about 3 times more Fan type and 23 times more Bambusoid class opal phytoliths than the amount of Fan type opal phytoliths produced by *Miscanthus* leaves. When compared to the amount of Panicoid class opal phytoliths of *Miscanthus* origin, *Pleioblastus* leaves produce 0.3 times more Fan type and 1.4 times more Bambusoid class opal phytoliths.

The results show that generally, production of non-short cell opal phytoliths such as Jigsaw-puzzle shape from *Fagus crenata*, and Fan shape (bulliform cells) from *Zoysia japonica* and *Miscanthus sinensis* are not as stable as of short cell phytoliths. It can be particularly well observed in the extremely low and uniform NV of the short cell phytoliths (e.g. Chloridoid/Bambusoid and Panicoid/Bambusoid) (Table 1). Blackman and Parry (1968), Kondo (1995), observed that opal phytoliths in leaves which are placed over the veins (costal) (e.g. dumbbel, saddle and cross shaped forms) occur invariably, while the ones located between the veins (intercostal) (e.g. bulliform and elongated forms) are ramdom and sporadic in occurrence. Furthermore, during the preparation of the samples, special care should be paid especially during the process of dispersion of the phytoliths. Since different portions of leaves carrying different rates of phytoliths are assembled, which may result in misinterpretation. Thus, the concave and convex shapes of curves observed in the results with increasing mixing ratio are probably due to problems in the dispersion of the particles during the preparation of the samples. Kondo (1988), also showed that some species show marked difference between theoretical estimation and practical counting values. Although Bulliform phytoliths are comparatively easier to identify, in this study it was observed that their use in interpretation of opal phytolith assemblage of soils should be restricted to qualitative (pres-

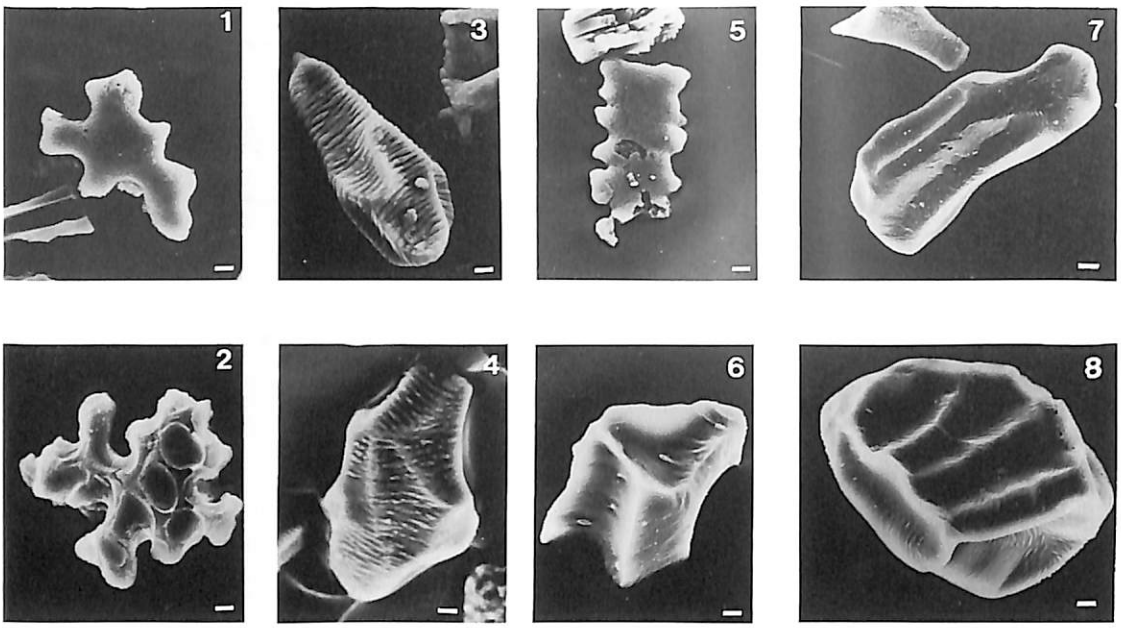

Plate 2 Scanning electron microscope photographs of phytoliths from trees
Fagus crenata : anticlinal epidermis (jigsaw-puzzle), 1, 2, *Persea thunbergii* : tracheid cells, 3, 4, *Picea ghlenii* : elongate sinuous, 5, transfusion tracheid, 6, *Castanopsis cuspidata* : elongate multifaceted, 7, spherical multifaceted, 8. Scale bar=5μm

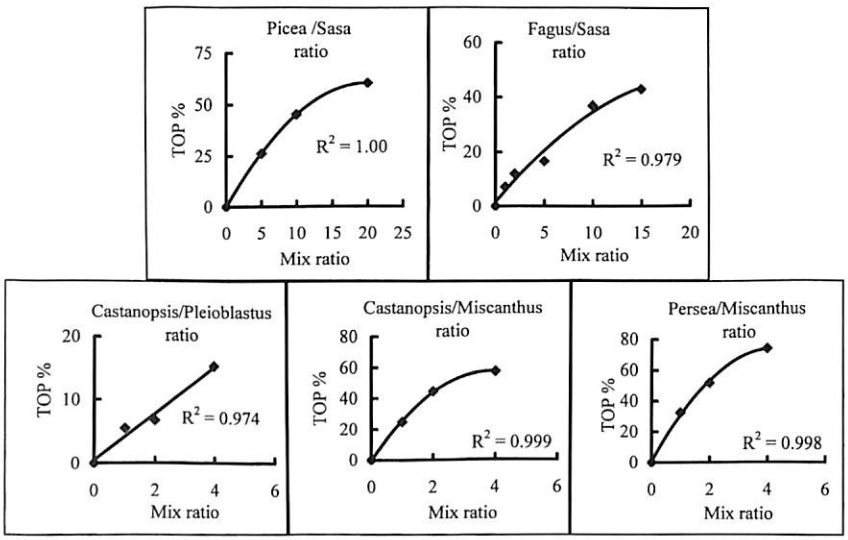

Fig. 4 Tree and grass origin opal phytolith ratios
TOP : percencentage of tree origin opal phytoliths, mix ratio : quantity of tree leaves per 1 of grass. 800 phytoliths were counted per each plot. *Picea* : *P. ghlenii* (collected in Obihiro, Hokkaido), *Sasa* : *S. kurilensis* (collected at Fuji Bamboo Garden, Shizuoka), *Fagus* : *F. crenata* (collected in Towada, Iwate), *Castanopsis* : *C. cuspidata* (collected in Nago, Okinawa), *Pleioblastus* : *P. simonii* (collected at Fuji Bamboo Garden, Shizuoka), *Miscanthus* : *M. sinensis* (collected in Obihiro, Hokkaido), *Persea* : *P. thunbergii* (collected in Nago, Okinawa).

ence or not of a plant) rather than quantitative (dominance of a plant) interpretation. Since quantitative interpretation without a more detailed study of each species, might lead to misinterpretations. Thus the use of short cell origin phytoliths seems to be more reliable than that of non-short cell origin phytoliths. However, the important role of non-short cell phytoliths in identification of certain species can not be overlooked. Some plants such as *Sasa*, *Zoysia*, *Picea* and *Fagus* (Plate 1) are easily identified through their characteristic shapes such as the Fan shape and Jigsaw-puzzle shape phytoliths.

In further studies conducted with grasses (*Sasa kurilensis*, *Pleioblastus simonii*, *Miscanthus sinensis*, Plate 1) and trees (*Picea ghlenii*, *Castanopsis cuspidata*, *Persea thunbergii*, *Fagus crenata*, Plate 2), the relation of their opal phytolith ratios was determined (Fig. 4). For this study, the total number of phytoliths (including sclereid, tracheid and epidermal phytoliths) from trees and grasses were determined. Through the results obtained the difference of opal phytoliths production between species became clear. Furthermore, trees produce much fewer opal phytoliths when compared to grass species. The result shows that to obtain similar amounts of tree and grass origin opal phytoliths in soils, a larger amount of tree leaves is necessary. Especially for *Castanopsis cuspidata*, *Fagus crenata*, *Picea ghlenii*, in relation to *Sasa kurilensis* and *Pleioblastus simonii*, the quantity of tree leaves should be more than 10 times of that of grasses in order to obtain the same amount of opal phytoliths. In case of *Castanopsis cuspidate* and *Persea thunbergii* in relation to *Miscanthus sinensis*, the ratio drops to about 2 : 1 (tree leaves : grass leaves).

IV. Conclusions

Through this study it became clear that the determination of phytoliths ratios between different species and their different shapes is a good tool to obtain an effective estimation of vegetation reconstruction through opal phytolith assemblages. The unstable production of Fan shape phytoliths by plants observed in this study, also suggests that the use of short cell opal phytoliths seems to be more reliable than the Fan shape phytoliths.

This study shows that although phytolith assemblage has been used to determine past environments, for a more accurate determination of vegetation change and paleoenvironment reconstruction, more detailed studies of the ability of different plants to produce opal phytoliths are required.

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(J+E) in Japanese with English abstract, (J) in Japanese.

植物珪酸体組成と植物生体量との関係

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〔摘 要〕

現状における植物珪酸体分析法では，過去の環境の正確な再現はまだ困難である．本論文では，植物珪酸体組成と植物生体量の関係について研究した．葉身を各種の比率で混合することによって，いくつかのイネ科草本および樹種の植物珪酸体生産性の差異を検討した．すべての植物は植物珪酸体の生産性にいくらかの変動を示したが，特に機動細胞珪酸体の生産性の偏差は大きかった．本研究の結果によれば，ブナの葉身によって生産されるジグソーパズル型珪酸体の量と比べて，チシマザサの葉身は23倍も多くのファン型珪酸体と27倍も多くのタケ型珪酸体を生産した．一般に，土壌中で樹木起源および草本起源珪酸体の量が等しくなるためには，樹木の葉量は草本の葉量の10倍以上必要である．供試植物種について得られた明瞭な結果から，各植物種の間で珪酸体の量比を求めることが植物の生体量を推定し，ひいては過去の植生をより正確に復元するための良い手段となることを示した．

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