Chemical composition and distribution pattern of crystalline inorganic components in Japanese gymnosperms

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Summary. It was found that the distribution pattern of calcium oxalate monohydrate present in the leaves of gymnosperms growing in Japan is classified into 2 groups by the technique of the 'low-temperature plasma ashing method for biological tissues'.

In order to establish the genealogical tree that reflects the distribution pattern of crystalline inorganic components in plants, systematic examinations are being made on the pattern of crystalline inorganic components in vascular plants growing in Japan[2–6]. In the present series, gymnosperms were taken up, and the chemical composition pattern of crystalline inorganic components in their leaves were examined. The gymnosperms examined constituted 8 families, 27 genera, and 55 species. Analysis of crystal pattern was made by the 'low-temperature plasma ashing method for biological tissues' that the present writer had devised, with which biological tissues are completely ashed on a glass microscope slide at low temperature by oxygen plasma gas in a high frequency electromagnetic field, preserving mineral microstructures precisely identical with the original tissue matrices. The following conclusions were drawn.

All the gymnosperms examined contained crystalline inorganic component which was limited to calcium oxalate monohydrate [$Ca(COO)_2 \cdot H_2O$]. Therefore, Japanese gymnosperm is a unified group of plants considering the chemical composition of their crystalline inorganic component.

Crystal form of the gymnosperms was discriminated into 3 types. Relationship among these 3 types and distribution of the types suggest classification of Japanese gymnosperms into the following 2 groups.

1. Cycadaceae-Ginkgoaceae series. This group of plants contain clusted crystals in the form of spiked ball. This group includes Cycadaceae and Ginkgoaceae. 2. Conifers

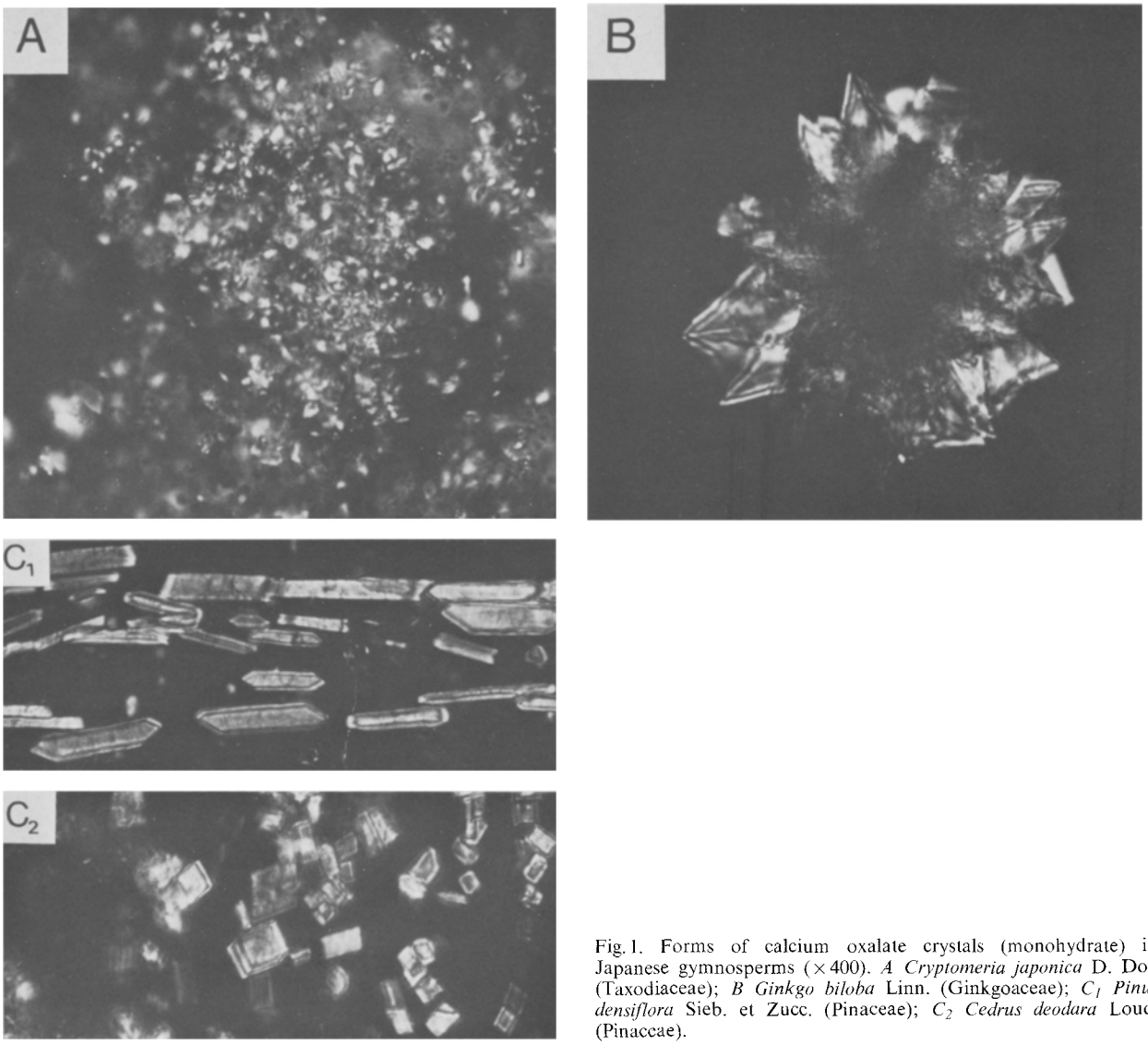

Fig. 1. Forms of calcium oxalate crystals (monohydrate) in Japanese gymnosperms (×400). *A Cryptomeria japonica* D. Don (Taxodiaceae); *B Ginkgo biloba* Linn. (Ginkgoaceae); C_1 *Pinus densiflora* Sieb. et Zucc. (Pinaceae); C_2 *Cedrus deodara* Loud. (Pinaceae).

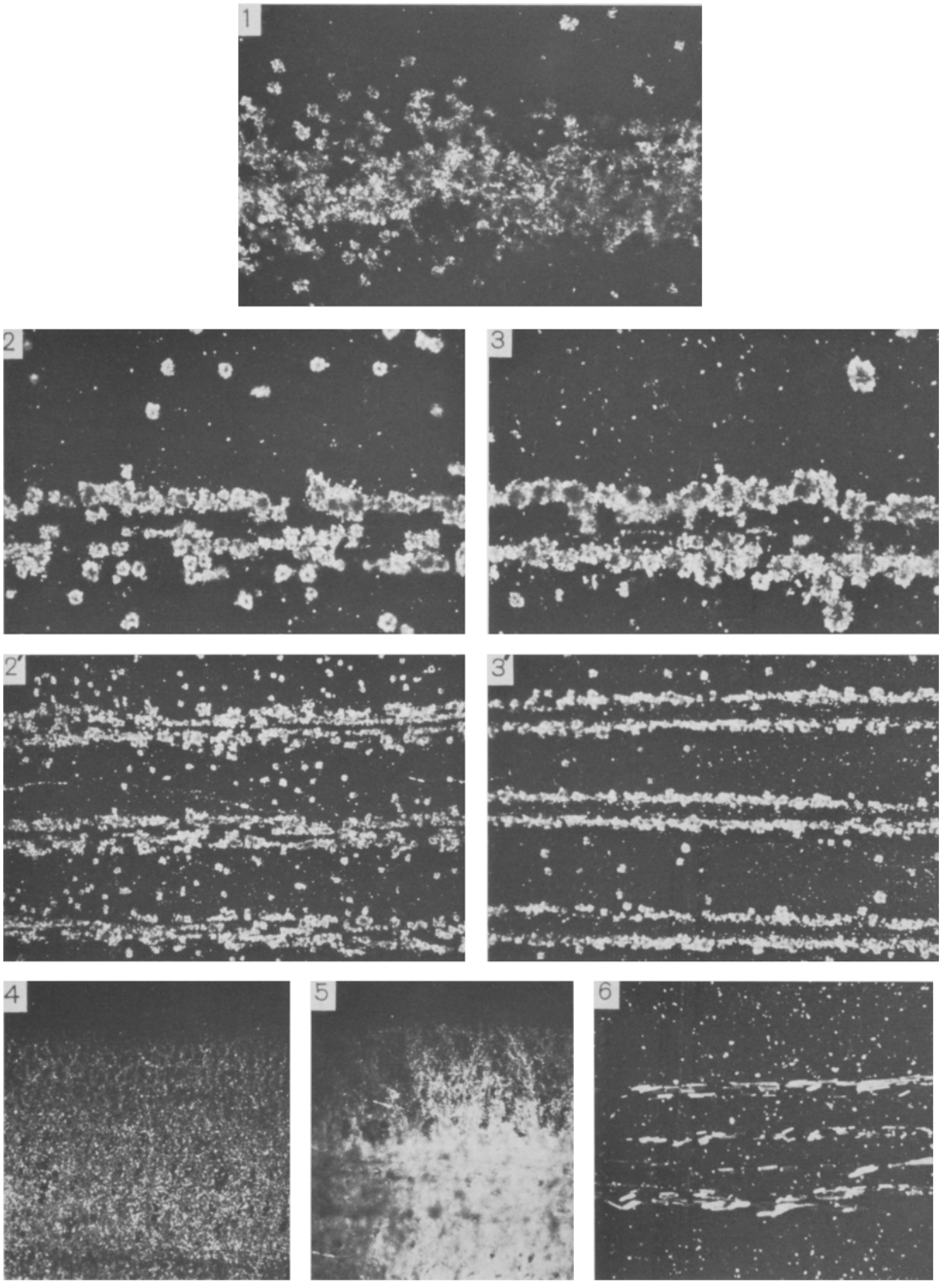

Fig. 2. Patterns of calcium oxalate crystals (monohydrate) in the leaves. *1 Cycas revoluta* Thunb. (Cycadaceae), × 100; *2 Zamia furfuracea* Ait (Cycadaceae), × 100; *2 Zamia furfuracea* Ait (Cycadaceae), × 40; *3 Ginkgo biloba* Linn. (Ginkgoaceae), × 100; *3 Ginkgo biloba* Linn. (Ginkgoaceae), × 40; *4 Cryptomeria japonica* D. Don. (Taxodiaceae), × 40; *5 Sciadopitys verticillata* Sieb. et Zucc. (Taxodiaceae), × 40; *6 Pinus densiflora* Sieb. et Zucc. (Pinaceae), × 100.

series. The crystal type found in this series is a collection of fine crystals of less than 5 μm, and these fine crystals are sometimes accompanied with square or rectangular solitary crystals. This group includes all Japanese conifers, i.e., Taxaceae, Podocarpaceae, Cephalotaxaceae, Taxodiaceae, Cupresaceae, and Pinaceae.

Japanese gymnosperms, therefore, are divided into 2 large systems from their crystal form. Considering the embryological evidence on the crystal pattern, the pattern of the evolution advances in the direction of a fine crystal type → a solitary crystal type. Therefore, Pinaceae plants which contain a large quantity of solitary crystals seem to be a more advanced group of the conifers. The crystal patterns of *Abies* and *Tsuga*, which contain a large number of fine crystals but only a few solitary crystals, are transitional between the crystal patterns of *Cedrus* and *Pinus*, which contain a lesser number of fine crystals and a considerable quantity of solitary crystals, and the crystal patterns of Taxodiaceae and families other than Pinaceae in the conifers. This evidence suggests that evolution advances in the direction of conifers (excluding Pinaceae) → *Abies* and *Tsuga* → *Cedrus* and *Pinus*. However, these points require further examination, with reference to other characteristics, before a definite conclusion can be drawn.

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Metabolic studies of Hg-203 on *Chlamydomonas reinhardi*[1]

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Summary. Sterile cultures of *Chlamydomonas reinhardi*, WT^+, were treated with Hg-203 at 25 °C to identify probably formed volatile mercury compounds. Experiments were performed with living and dead cells under aerobic or anaerobic conditions, respectively, and the mercury concentration was measured in the system algae/nutrient medium. We found a timerelated decrease of mercury concentration in the cell suspension and the cell-free nutrient medium due to a reduction of Hg^{++} to Hg^0, probably caused by extracellular enzymes; monomethyl or dimethyl mercury could not be detected.

The increasing application of toxic elements in industry and agriculture causes environmental pollution and human diseases. Especially the highly toxic metal mercury attracts considerable attention on account of the accident in Minamata, Japan[3,4] and the effect of rather careless use of methyl mercury-containing seed dressings in Sweden[5–8]. Therefore extensive research has been performed on the fate of mercury in food chains; it could be shown that microorganisms are able to metabolize mercury compounds[9–15]. This reaction, regarded as a detoxification mechanism[16], can yield a product that is either more or less toxic to higher organisms. Ben-Bassat et al.[17] exposed *Chlamydomonas reinhardi* to mercury (II)-chloride and found a decrease of mercury concentration in the cell suspension; they supposed metabolization to volatile compounds, e.g. methyl mercury. Our work focused on the identification of probably formed volatile mercury compounds.

Materials and methods. Algae: The unicellular algae *Chlamydomonas reinhardi* was the organism employed in this work. Slant-agar stocks of WT^+ (wild type) were kindly given by R. Davies (John-Innes-Institute, Norwich, England). Pure cultures of the cells were grown asynchronously, i.e. by means of continuous illumination, at 25 °C and air bubbling through (1.5 l/h). Cultivating was done in 500 ml Erlenmeyer flasks containing 200 ml YAP[18] under sterile conditions; the final cell concentration was 5.10^6/ml (determinded by means of Thoma counting chambre).

Mercury: Mercury-203 was used as nitrate. This isotope was obtained by neutron activation (flux = 4.10^{13} n cm^{-2} sec^{-1}) of metallic mercury (analytical grade from Merck, Darmstadt, Federal Republic of Germany) in the ASTRA-reactor of the Research Centre Seibersdorf, Austria. After treating with concentration HNO_3 aqueous $^{203}Hg(NO_3)_2$ standard solutions were prepared (specific activity 1.9 μ Ci/ml). The initial concentration used in experiments was $8.2 \cdot 10^{-7}$ moles/1.

Measurement of mercury concentration in the system algae/nutrient medium. All experiments were performed at 25 °C under sterile conditions; the medium to be tested (cell suspension or YAP, respectively) was vigorously stirred and a stream of gas (air or nitrogen, respectively) was blown through it at a rate of 1.5 l/h. At certain time intervals 2 ml-samples were withdrawn, transferred into special counting tubes and measured on a single channel analyzer. In each case newly prepared sterilized YAP treated in the same way was used as control.

Cell suspension: The time-related decrease of mercury concentration in the cell suspension was studied on living and dead cells under aerobic (light/air (LA)) and anaerobic (dark/nitrogen (DN)) conditions as well. For DN-experiments using living cells 'preconditioning' (i.e. keeping under conditions of the following experiment) of cell suspension was done for 1 h before adding the heavy metal ion standard. In doing so respiration and photosynthesis were interrupted. Pre-conditioning was not necessary for LA-experiments. Experiments using dead algae required pre-conditioning for DN- and LA-experiments. Dead cells were obtained either by γ-irradiation (0.6 mrad/h, 5 h, ^{60}Co, Institute of Biology, Research Centre Seibersdorf, Austria) or steam sterilization (120 °C, 20 min).

Used YAP: The time-related decrease of mercury concentration was studied on the cell-free nutrient medium already used for cells grown until the end of their log-phase. 2 different methods were applied: 1. After centrifuging the living cells, both heat sterilized YAP (a) and non-sterilized