general breakup of Gondwanaland. As the nearest pole of rotation lies to the north, the spreading rate on the ridge that created the Cape Basin lineations must have increased towards the south. Thus, somewhere south of the Cape Basin this ridge met a triple junction, or was transformed to another spreading centre with a faster rate. It is unlikely that the ridge could have been transformed between Antarctica and South America into the Pacific because the Palaeozoic and Mesozoic formations on these continents form continuous sequences that were not fragmented into the Scotia Arc until at least the end of the Andean Orogeny in the Cainozoic[18].

Thus, the ridge was probably transformed to the east into the newly forming Indian Ocean or between the two stable masses that now form east and west Antarctica. The former suggestion is similar to that made by McKenzie and Sclater[19] for the plate boundaries in this area from 75 to 55 m.y. BP. It is an hypothesis that awaits testing by determining the presence or absence of the Mesozoic lineation pattern at the stable margins of the Indian Ocean.

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ROGER L. LARSON
JOHN W. LADD

Lamont-Doherty Geological Observatory of Columbia University, Palisades, New York 10964

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World-wide Source of Leaf-derived Freezing Nuclei

INITIATION of precipitation over much of the Earth's surface is believed to be intimately related to the development of ice in supercooled clouds. The source, composition and number of small particles (ice nuclei) initiating freezing events in natural clouds are poorly understood. These particles represent a very small fraction of the total aerosol in the atmosphere. The material most frequently held to represent the majority of ice nuclei are clays, especially kaolin.

In a summary of earlier research, we reported[1,2] that decomposed plant leaf litters contain large numbers of freezing nuclei and that these nuclei are perhaps responsible for the nucleating abilities of soil particles. (Freezing nuclei are defined as particles capable of nucleating ice in supercooled water. If no distinction is made between freezing and deposition the nuclei are referred to as ice nuclei.) The leaf-derived nuclei (LDN) were found to initiate freezing of supercooled water drops at temperatures warmer than $-5°$ C and to occur in concentrations of up to 10^{10} nuclei active at $-10°$ C per g of decaying leaf. The conclusion that the nuclei are products of decomposition was reached by following the decay of fresh leaves in the laboratory and observing the gradual evolution of nuclei to the stage comparable to naturally decomposed litters. (Extensive chemical analyses have not yet revealed the structure of the LDN but indicate that the nucleating particles probably constitute less than 0.1% of the total decayed leaf litter mass. Filtration tests suggest that the LDN particles are between 0.1 and 0.05 μm in diameter. Heat in excess of 60° C deactivates the nuclei, supporting the suggestion that they are organic in nature.) Though it has not yet been shown that the mechanisms transferring LDN to the atmosphere are of sufficient intensity to account for atmospheric ice nucleus concentrations, it was felt important to establish the extent to which LDN are available on a global basis.

We collected samples of well decayed litter from fifty different dominant tree or grass species across the northern hemisphere. The sampling locations were spread throughout North America, from the Pacific to the Atlantic, and across Europe and northern Asia from Britain eastward through Siberia to Japan. A second set of samples was collected along a transect from Japan through southeast Asia into western India.

Testing of the little samples for freezing nucleus content was done in a standardised manner by inserting 1 g (dry weight) of leaf material into 100 g of distilled water followed by agitation and a 5-min settling period. The resulting suspension was filtered through a paper filter with a pore size of approximately 50 μm. The slightly turbid filtrate was tested on a nucleus spectrometer following the principles and procedures outlined by Vali[3].

Numerous samples were found to contain freezing nuclei in numbers comparable with what has been reported earlier[2]. Some other samples were found to contain fewer ice nuclei, by factors of up to 10^5. The abundance of nuclei in a sample appeared not to be correlated with species but showed a closer similarity to samples from the same geographical location. On closer analysis it became apparent that nucleus content is closely related to the climate prevailing at the sample's origin, almost regardless of the species of plant.

According to Köppen[4], the Earth is divided into six principal climatic zones depending on seasonal temperature and variation, precipitation pattern, and physiography. Of the six zones, three have extensive vegetation (A, tropical; C, humid mesothermal; D, microthermal) and the remaining three have lesser amounts of vegetation (B, dry; E, polar; H, undifferentiated highlands). The type of vegetation growing in each climatic zone is very distinctive for that zone. Data are presented in Fig. 1 for three climatic zones for which a minimum of twelve nucleus spectra per zone is available. For each climatic zone, the highest, lowest and median spectra are plotted. A clear separation between the three zones is evident.

Samples of the genus *Poa* were collected from each of the climatic zones A, C and D. The nucleus contents of the three samples were found to coincide with the typical spectra for each of the zones. Samples of the genera

Fagus, Oryza, Populus and others were available from two of the climatic zones. In each case the nucleus content followed the climatic trend. Thus, ice nucleus content can be clearly seen to depend primarily on the climate of a sample's origin; it is also evident that the range of nucleus contents for samples from a given zone is quite restricted.

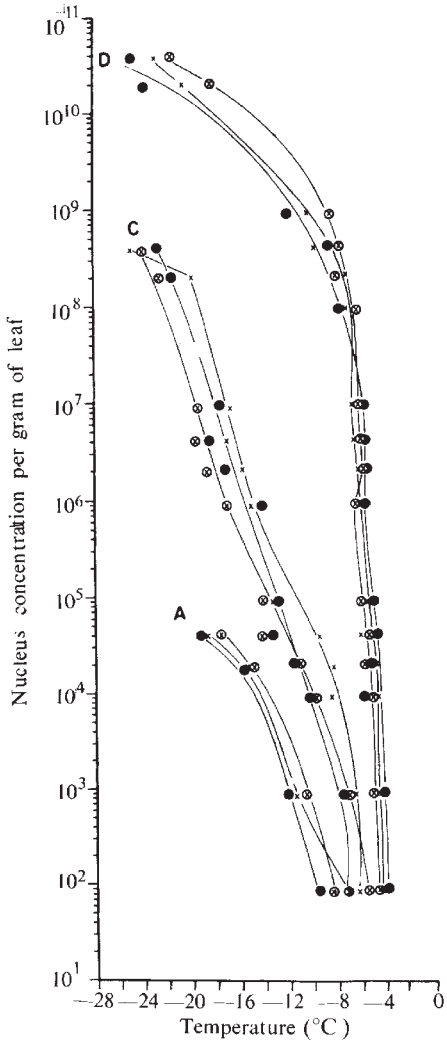

Fig. 1 Freezing nucleus spectra of well decayed leaf litters from A, C and D-type climate zones. For each zone, the highest, lowest and median spectra are plotted.

What factors cause the close tie between nucleus content and climate of growth? Since the process of formation of the freezing nuclei from leaf matter is not yet known, the reasons can only be speculated upon. It is known in general that decomposition is mediated by bacteria and the specific role of bacteria in producing freezing nuclei from leaf matter was demonstrated by Fresh[5]. Differences in microflora among climatic regions might thus be one of the factors. Others of possible importance are the availability of moisture, average air temperature and type of soil substrate.

The climatic dependence of freezing nucleus content in leaf litters does not make itself evident in the concentrations of airborne ice nuclei which have been found to be fairly constant globally[6]. Atmospheric mixing and the possibility that the release of nuclei from litters may also be variable with climate, in an inverse way compared with the availability of nuclei, may account for the observations.

In summary, the world-wide availability of LDN strengthens the suggestion that LDN may constitute a significant fraction of the atmospheric ice nuclei.

R. C. SCHNELL
G. VALI

Department of Atmospheric Resources, University of Wyoming, Laramie

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BIOLOGICAL SCIENCES

Rapid Screening Assay for Revertants derived from MSV-transformed Cells

MURINE sarcoma virus (MSV)-transformed 3T3FL cells (S^+L^- cells[1]) produced spontaneously and at variable rates flat variants (revertants) from which MSV could no longer be rescued by superinfection with murine leukaemia virus (MuLV)[2,3]. During the course of investigation of reversion frequency in S^+L^- cells, we have been able to detect revertants by a rapid screening assay technique instead of using the more cumbersome cloning procedure in microtitre wells as previously described[2,3]. The S^+L^- cell line 3197[1-3] was composed of hyper-refractile, loosely attached cells and did not form a compact cell monolayer. Infection of this cell line with MuLV changed the cellular morphology to a slightly more rounded cell type, and increased the tendency of cells to detach readily from the surface of the container. When rescue-negative revertants derived from S^+L^- cells were infected with MuLV there was no observable change in cellular morphology. These characteristics can be used as the basis for a rapid screening assay technique for revertants from S^+L^- cells, the details of which are described here.

The sublines of 3197 cells, 3–321 and 3–360 (ref. 4), grown in modified McCoy's 5a medium with 10% foetal calf serum (Grand Island Biological Co.) were infected with the IC isolate of Moloney leukaemia virus[5] at MOI>5 focus inducing units (FIU)[6], and 1,000–5,000 cells in 5 ml medium were delivered into 25 cm^2 flasks (Falcon). Flasks were incubated at 37° C for 4 d, then shaken to detach the relatively loose S^+L^- cells; supernatant was removed and flasks were washed twice with medium. Five millilitres of fresh medium were added and flasks were further incubated at 37° C. After a further 3–5 d of incubation, flat, contact-inhibited revertant cell colonies formed and were counted. When S^+L^- cells were infected with MuLV at high MOI (>5 FIU), MSV was rescued from them about 12 h after infection[7]. At that time, viral interference had developed in rescue-negative evertant cells following MuLV infection, and no transformation of revertants was produced by the rescued MSV. Therefore, revertants remained flat and formed colonies.

The sensitivity of this technique in the detection of revertants was compared with that of cloning techniques[2,3] in which flat cell colonies were isolated in microtitre wells (Micro Test II tissue culture plate, Falcon) and then tested for rescuable MSV genome by superinfection with MuLV[1-3,7]. Quantitatively, these two assays gave results which were in close agreement, with the following reversion frequencies: 0.0002 and 0.0003 in 3–321 cultures, and 0.003 and 0.005 in 3–360 cultures, as can be seen in Table 1.