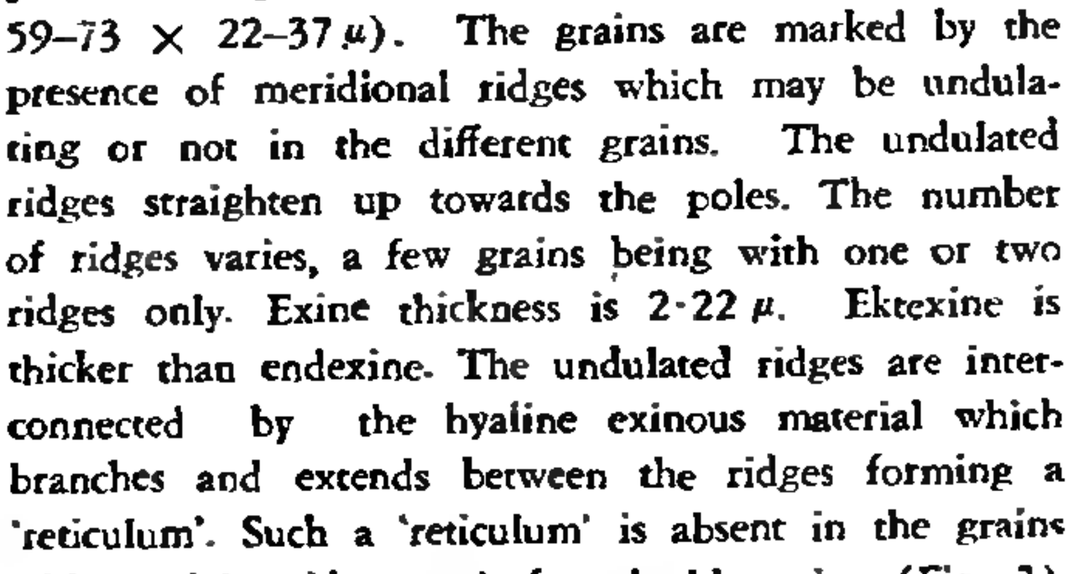
POLLEN GRAINS IN *EPHEDRA HELVETICA* C. A. MEY.

AMONG the Gymnosperms, *Ephedra* occupies a unique position, in all aspects of its biology, reflected amply in pollen morphology alone, having inaperturate grains possessing meridional ridges, an evidence of a higher level of morphological evolution. Being characteristic in its morphology, the identification of fossil pollen of *Ephedra* has been comparatively easy, and provides a sure index in interpretations of palaeoclimate and phytogeography.

Steeves and Barghoorn[1] made a monographic investigation of the pollen morphology of 43 species of *Ephedra*. They contended that the grains of *Ephedra* are polycolpate, considering that the areas between the ridges represent the furrow areas. In the background of the above finding the present report on the pollen grains of *Ephedra helvetica* C. A. Mey. is made.

The pollen material of the species has been procured from the Royal Botanic Gardens, Kew, England, and pollen preparation has been made by the acetolysis method[2]. The pollen grains of *E. helvetica* are inaperturate (Fig. 1) as a general rule; a few 1–colpate grains (Fig. 2) also occur. Average diameters of the polar and equatorial axes are 64 × 29 μ (range 59–73 × 22–37 μ). The grains are marked by the presence of meridional ridges which may be undulating or not in the different grains. The undulated ridges straighten up towards the poles. The number of ridges varies, a few grains being with one or two ridges only. Exine thickness is 2·22 μ. Ektexine is thicker than endexine. The undulated ridges are interconnected by the hyaline exinous material which branches and extends between the ridges forming a 'reticulum'. Such a 'reticulum' is absent in the grains with straight ridges. A few double-grains (Fig. 3) with straight ridges are also seen in the species as an abnormal feature.



FIGS. 1–3. Pollen grains of *Ephedra helvetica*. Fig. 1. Inaperturate grain. 2. 1–colpate grain, 3. Double-grain. Magnification : × 500.

Among the modern *Ephedra* the occurrence of clearly defined 1–colpate grains in *E. helvetica* raises doubts about earlier interpretations of polycolpate condition in its pollen as envisaged by the thinning or absence of ektexine between the ridges[1]. For *E. funera*, Steeves and Barghoorn[1] have presented a photomicrograph of the polar view of a grain. The regions between the ridges show a well developed exine and give no way to be called as furrows or colpi as stated by the authors. The thickening of ektexine at the ridges may be given only a structural significance.

A well marked colpus has earlier been reported by Kuyl *et al.*[3] in *E. strobilacea* type grain with ridges, from Cretaceous sediments in Iraq. In that light, the occasional occurrence of 1–colpate grains in modern *E. helvetica* has an evolutionary significance. This, being a primitive character, may also indicate the possible remote phylogenetic affinity of the genus and the group (Ephedrales), with the Cycadales.

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RAPID DETECTION OF INDOLE COMPOUNDS WITH EHRLICH'S REAGENT

EHRLICH'S reagent[1] consisting of an acidic solution of *para* dimethyl aminobenzaldehyde (PDAB) has been employed for the quantitative estimation of various aromatic compounds[2-4]. During the studies relating to the enumeration of soil and rhizosphere microflora producing indole acetic acid, it is observed that a 10% w/v solution of PDAB in perchloric acid reacts with indole acetic acid forming a pink colour instantaneously. The colour complex is stable for 10 min, shows an absorption maximum at 565 mμ and gives linearity with concentration in the range of 2–6 μg.

For determinations, 4 ml of the PDAB reagent is added to 1 ml of an aqueous solution containing indole acetic acid and the intensity of the coloured complex measured at 565 mμ. The method is superior to the conventional salkowsky[5] in rapidity, but is non-specific for indole acetic acid. The reagent forms colour complex with indole (λ_{max} 430 mμ), indole acetic acid (λ_{max} 565 mμ), indole pyruvic acid (λ_{max} 560 mμ), indole propionic acid (λ_{max} 585 mμ), indole butyric acid (λ_{max} 570 mμ) and indole lactic acid (λ_{max} 580 mμ).

Bacterial metabolism of tryptophan is characteristic of its degradation to indole and pyruvic acid with the liberation of ammonia. Micro-organisms can as well breakdown the side chain of tryptophan forming indole pyruvic acid and indole acetic acid. The classical method employed for qualitative detection of indole is the Kovac's test[6], employed for the classification of coliform bacteria[7]. The indole acetic acid and indole pyruvic acid however do not respond to Kovac's reagent[6]. The modified PDAB reagent in perchloric acid may be used for the qualitative detection of the different substituted indole compounds including indole acetic acid and indole pyruvic acid. Detection of indole acetic acid with the Salkowsky reagent[5] is time consuming while indole pyruvic acid poorly responds to the reagent.

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STIGMATIC EXTRACTS OF *CHLOROPHYTUM HEYNEANUM* ENHANCE *IN VITRO* GERMINATION OF *C. MALABARICUM* POLLEN GRAINS

In vitro germination requirements of pollen grains vary. The pollen of some species are able to germinate in distilled water while a number of others require simple or mineral supplemented sugar solutions. In other cases, pollen grains easily germinate *in vivo* but fail to do so in culture media. Addition of stigmas to the culture medium may initiate pollen germination under such circumstances[1]. As for *in vivo* germination, stigmas generally support development of functional pollen of the same species but not of alien species[2]. However, we found that the pollen of *Chlorophytum malabaricum*, although inert on its own stigma, germinated easily on the stigma of *C. heyneanum*. Furthermore, stigmatic extracts of *C. heyneanum*, added to culture medium, brought about marked improvement in the rate of pollen germination of the other species.

C. heyneanum is male fertile with an average *in vitro* germination of 90%. The other species, *C. malabaricum*, bears short styled totally male sterile as well as long styled partially sterile flowers with an average of 30% germination *in vitro*. Only the latter type of flowers of this species was used in this study. As in other plants, *Chlorophytum* also shows flower to flower and even anther to anther variations in pollen germination. Discrepancies on this account have been minimised by mixing pollen grains from 20–25 flowers and taking samples from the same mixture.

In vivo germination was studied under laboratory and also field conditions. Germination was checked 2–3 hr after pollination by viewing unstained and cotton blue-stained stigmas under the microscope. Post-pollinated stigmas were also observed periodically up to 12 hr to study the development of pollen tubes.

The standard medium containing sugar, calcium and boric acid was used as controls and for trials ethanolic or distilled water extracts of twenty stigmas prepared according to the method of Namboodiri and Tara[3] were added to the medium. Two-dimensional chromatograms of the stigmatic extracts with BAW and Acetic Acid as solvent systems were developed and spot tests were conducted according to standard techniques.

A summary of pollen germination percentages under various conditions is given in Table I. *C. heyneanum* pollen germinate in standard medium in the range of 80–96%. Addition of alcohol to the medium inhibits the rate of germination by as much as 30%. No significant effect on the rate of *in vitro* germination occurred with the addition of stigmatic extracts — either of its own or that of *C. malabaricum*—to the medium.

The *in vitro* rate of germination of *C. malabaricum* is relatively low (20–35%). As in *C. heyneanum*, alcohol has an inhibitory effect on *in vitro* pollen germination of this species. Pollen of *C. malabaricum*