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IMPROVEMENTS IN THE SQUASH TECHNIQUE FOR PLANT CHROMOSOMES

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It is difficult to find a chromosome squash and mounting technique which adequately meets the requirements necessary for mounting chromosomes of plants having PMC's which are few in number, small in size, and adhesively retained in small anther sacs. These perplexing attributes are exhibited by *Zinnia* in the family Compositae, *Gilia* in the family Polemoniaceae, and especially *Allophyllum*, also in the Polemoniaceae. Not only are the PMC's of these genera difficult to liberate from the anther sacs but the usual permanent mounting techniques, at times, have not been satisfactory. Either the arduous and time-consuming alcohol to diaphane diffusion method (Bradley, 1948) was used or the cover slip had to be removed (McClintock, 1929) which in many cases resulted in the loss of desired material. When the PMC's were mounted in the alcohol-soluble mediums, there was always the risk that the staining of the chromosomes might fade or be masked by excessively stained cytoplasm.

The liberation of PMC's from the anther sacs has been satisfactorily accomplished by first soaking the anthers in the stain for fifteen seconds, then macerating them in 5% HCl for thirty seconds to two minutes at 25 degrees C. The HCl was drawn off with filter paper and then the anther crushed and stained. Care should be taken not to allow the anthers to remain in the HCl any longer than is necessary or the chromosomes may become difficult to stain.

The problems of permanent mounting have been alleviated greatly by the use of the water-soluble Hoyer's mounting medium; this medium was introduced to me by Dr. Richard K. Benjamin, Mycologist, Rancho Santa Ana Botanic Garden.[b] The Hoyer's medium allows the technician to squash and permanently mount the chromosomes in a single process. In 1950, while at the University of Illinois, Benjamin obtained the formula for Hoyer's medium from Lewis J. Stannard, Jr., Illinois Natural History Survey, Urbana, Illinois. At the time the medium was being used to mount small insects and arachnids. Benjamin, however, found the medium to be excellent for whole mounts of the members of the order Laboulbeniales in the class Ascomycetes, mosses and liverworts, and the spores and capillitia of the Myxomycophyta. Anderson (1954), also reports the use of Hoyer's medium for Bryophytes.

It is believed that probably there are many more uses for this versatile medium. Recently Benjamin found that by varying the fixative, Hoyer's medium could be used for making chromosome mounts of several algae, e.g. *Cladophora, Oedogonium,* and *Volvox.* It is suspected also that Hoyer's medium might shorten the laborious secondary wood section mounting methods. At present I am endeavoring to work out mounting schedules for the material above as well as for macerated root tips and ovules.

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[b]The work reported in this paper was done while technical assistant to Dr. Verne E. Grant, Rancho Santa Ana Botanic Garden.

The lasting qualities of the materials mounted in Hoyer's medium are extremely attractive. Calcareous crystals, as shown in Benjamin's four and one-half year old specimen mounts of the order Physarales in the Myxogastres, still are in excellent condition. Twenty year old U. S. National Museum insect mounts show no deterioration (Anderson 1954). Approximately one hundred chromosome slides which I made a year ago are as well preserved today as they were the first day they were mounted. The chromosomes have not faded, and there is a decided tendency for the cytoplasm to clear.

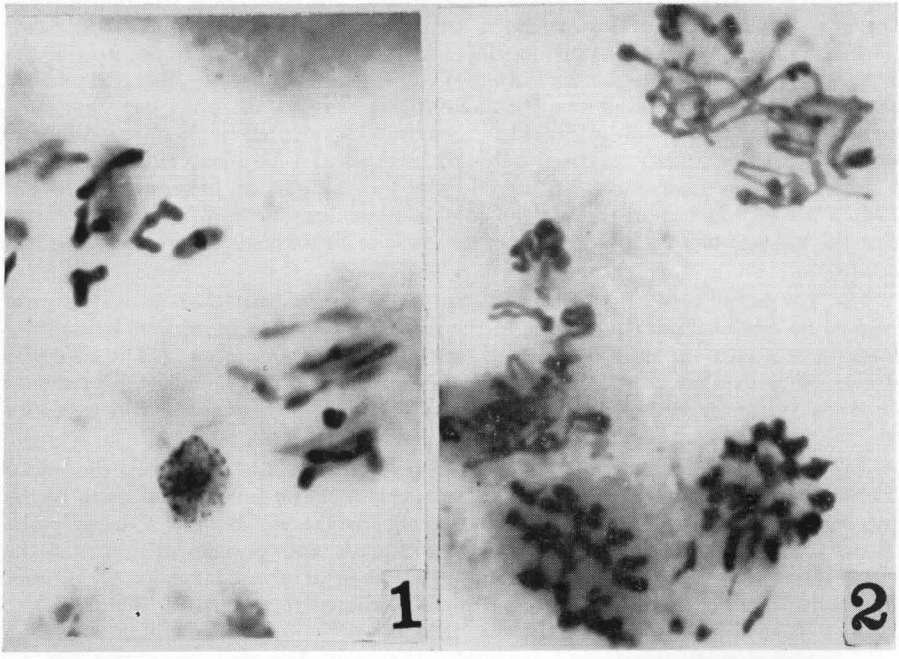

Fig. 1. *Allophyllum divaricatum* PMC's in MI. Fig. 2. An aberrant sporad in a Gilia hybrid. The above two figures are shown only because the material is exceptionally difficult to work with, not because the results are spectacular.

Samples of PMC's from seven genera: *Zinnia, Gilia, Allophyllum, Cymbidium, Argemone, Rhoeo, Paeonia,* and *Secale,* have been mounted successfully in Hoyer's medium by using two procedures.

The schedule is as follows:

I. Squash and Mount
   A. Stain and apply the cover slip; but before squashing, heat the slide over a steam bath, microscope lamp or warming plate rather than above an alcohol flame.
   B. Squash, then ring the cover slip with a sealing medium of 10 gms. gelatine powder: 100 ml. 50 per cent glacial acetic acid (Darlington, LaCour 1950).

C. When permanent mounting is desired, float off the cover slip with 50 per cent glacial acetic acid and immerse both the cover slip and slide in distilled water until the excess stain has diffused from the slide; time, approximately one minute.

D. Remove the slide and cover slip, wipe away the surplus water from around the stained area and apply a small drop of Hoyer's medium in the center of the area.

E. Replace the cover slip and heat over steam until the medium spreads to the edge of the cover slip.

F. Gently press on the cover slip to exclude the excess medium.

G. Harden the mount by placing the slide on a warming plate, 26 degrees C. A cover slip weight will help decrease the depth of field.

II. Permanent Squash

A. After crushing the anthers, apply a small drop of stain 2-3 mm. in diameter. Work slightly less iron than usual into the stain. The staining time will vary in accordance with the material being used and the use or omission of the maceration technique. However, do not allow the stain to remain exposed to the air long enough to cause precipitation.

B. With a thin needle drop a small amount of Hoyer's medium into the center of the stained area.

C. Refrain from disturbing the surface of the slide; the medium tends to settle and flatten the cells immediately. However, the stain and medium should be mixed *briefly.*

D. Apply the cover slip and heat over steam or on a microscope lamp until the medium flattens (a few seconds), then wipe away the water condensation on the slide.

E. Invert the slide on a disc of filter paper; fold the filter paper over the slide. While steadying the slide with one hand, *slowly* apply the squash with the other. (Small PMC's require a great deal of pressure. The amount of force is inversely proportional to the increase in cell size.) Release the pressure slowly so that the cell membranes will not be torn.

F. Examine the slide under a microscope. If the cells are not spread as desired, invert the slide over the steam bath for 3 or 4 seconds and repeat the squash.

G. Place the slide on a warming plate for curing. (This step tends to clear the cytoplasm, darken the chromosomes, and seal the slide.)
The slide may be cleaned with a damp rag.

Permanentizing in Hoyer's medium has two decided advantages:

(1) If for any reason the staining quality of the slide is unsuitable, the cover slip may be removed by inverting the slide over a steam bath and the material restained or destained as required.

(2) If at times air bubbles become trapped under the cover slip, they can be expelled by inverting a cured slide over steam.

Composition of Hoyer's mounting medium (Alexopoulos and Beneke 1952):

1. Distilled water..... 50 ml.
2. Arabic gum (lump) ..... 30 gms.
3. Chloral hydrate.....200 gms.
4. Glycerine ..... 16 ml.

Completely dissolve the Arabic gum in the water and in this solution dissolve the chloral hydrate. Each of these processes may take up to twenty-four hours. Add the glycerine and mix well. Due to evaporation the medium, at times, may become too concentrated and cause plasmolysis and tearing of the PMC's, or precipitation of the stain. Water may be added to thin the medium to the desired consistency.

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