SCIENTIFIC APPARATUS AND LABORATORY METHODS

A BLEACHING AND CLEARING METHOD FOR PLANT TISSUES

FOR the study of floral anatomy, the importance of which in tracing out the intricate evolutionary interrelationships of the angiosperms has long been recognized, the classical method is the very accurate but slow and laborious one of reconstructing the anatomical pattern from a long series of prepared sections. The drawback of this method is that time permits the study of only a few representative types, and only those species can be investigated of which fixed material is available to the worker. The following method, which was devised for the study of the anatomy of the florets of the Compositae, is not only so rapid that fifty or a hundred organs could be prepared for study within a week, but in addition it can be used equally well (or even better) on herbarium material, so that any species of which a herbarium specimen is available can be classified as to its type of floral anatomy.

The procedure is as follows: remove the flower or organ from the plant or dried specimen, and boil 2 to 3 minutes in water. Then place in a mixture of concentrated ammonium hydroxide (NH_4OH) and hydrogen peroxide (H_2O_2) of varying proportions, depending on the nature of the specimen. For relatively colorless objects 2 parts NH_4OH to 1 part H_2O_2 is best, but if the organ contains much chlorophyll, or if it is a badly oxidized, brown or blackish herbarium specimen, high concentrations of H_2O_2, up to 1 to 1 mixture, are necessary. For most objects 24 hours in this solution is sufficient, but for unusually opaque organs and badly discolored herbarium specimens the solution should after this time be renewed, and the specimen treated for a second or even a third day. Great care should be taken in pouring off the liquid, since the specimens are at this stage very soft and fragile. Then place in 95 per cent. alcohol and leave from 1 to 12 hours, depending on the time needed for the material to harden and the bubbles formed by the H_2O_2 to be driven out. Excess bubbles may then be removed by gently tapping the object or by pricking with a fine, sharp needle. Then place in n-butyl alcohol, using 3 changes at 1- to 2-hour intervals, then 1 to 1 mixture of butyl and xylol, 100 per cent. xylol, and mount in balsam. Staining with aqueous gentian violet (1 per cent.) is possible after hardening in alcohol, but most preparations can be studied equally well or even better without staining.

This method removes the cell contents and makes the cellulose walls transparent, so that the lignified protoxylem strands of the vascular bundles stand out sharply. It is ideal for small objects, like the florets of most Compositae, and can be used for organs up to about 2 mm in thickness, such as whole flowers of *Cornus,* although best results on relatively large specimens are obtained by preparing some whole and splitting other specimens of the same material down the middle, or by pressing them gently between a slide and cover glass after treatment with ammonia-peroxide, and hardening them on the slide by flooding it with alcohol. For unusually simple objects, like the ovaries at anthesis of *Lactuca* or *Taraxacum,* satisfactory temporary preparations can be obtained from dried ones that are not badly discolored by merely boiling them, first in water, then in concentrated ammonia. Difficulties are encountered with this method when the organ contains sclerenchymatous tissues, large secretions of oily or fatty substances, or a heavy coat of pubescence, although the latter can often be scraped away with a sharp scalpel from fresh, living specimens or from dried ones just after the preliminary boiling. While the method is obviously not suitable for working out the principles of floral anatomy, it is ideal for studying the distribution of the various anatomical types which have been worked out from sectioned material, so that they may be used as additional criteria in systematic and phylogenetic studies of genera and families of angiosperms. The following two examples illustrate the use of this method.

(1) *The stigma of some apocarpous dicotyledons.* The work of Eames[1] has definitely established the follicle as the most primitive type of carpel, while Hunt[2] has shown that certain types of stigmas clearly show the homology of the carpel with a foliar organ or sporophyll. The discovery, therefore, in a plant otherwise primitive of a follicle whose stigma contains an elaborate branch system is valuable evidence in favor of this theory of the carpel. Such a follicle and stigma exists in the genus *Paeonia,* while the branching type of stigma anatomy occurs in *Actea spicata* L., (Ranunculaceae), *Akebia quinata Decne* and probably all other *Lardizabalaceae,* and in *Uvaria purpurea* Blume of the *Anonaceae.* In the first three genera, the stigma is supplied by the ventral as well as the dorsal bundles, so that it is derived from the trilobate type of sporophyll (*cf.* Hunt, *loc. cit.,* Fig. 7). The branching system is the most elaborate in *Paeonia,* in which the dorsal bundle may have 8 successive bifurcations within the stigma, and the ultimate number of branchlets is in the hundreds. In *Actaea* and *Akebia* the branches are relatively much reduced and crowded. In *Uvaria* the broad stigma is supplied by the elaborate branching of the dorsal bundle, while the two ventrals end at the summit of the carpel proper with a few short branchlets, one of which fuses with the dorsal bundle. In the achene of *Hydrastis cana-*

[1] A. J. Eames, *Am. Jour. Bot.,* 18: 147–188, 1931.
[2] K. W. Hunt, *Ibid.,* 24: 288–295, 1937.

densis L. (*Ranunculaceae*), the stigma is fundamentally trilobate, but here the branching of the dorsal bundle is much reduced, while two or three major branches of each ventral divide again once or twice to produce the greater part of its vascular supply. In *Helleborus foetidus* L. the ventrals go part way up the linear style, where they fuse with the branched dorsal bundle, while in *Trollius laxus* Salisb. this fusion occurs at the summit of the carpel proper. In *Dillenia Reifferscheidia* Vill. (Dilleniaceae), the two ventrals each branch once and end in the middle of the broadened, but elongate style, while the dorsal continues to its end.

(2) *The ovary of the Cichorieae.* Although the anatomical pattern described by Koch[3] for *Lapsana* is identical with that of all other Cichorieae as regards the corolla, stamens and stigma, considerable variation has been found in the structure of the achenes, and this has proved a valuable aid in delimiting some of the genera of this complex group, and in determining the phylogenetic position of the species. It has already been used in distinguishing between *Lactuca* and *Ixeris,* which latter genus, though unquestionably distinct and actually more nearly related to *Crepis* as has been pointed out by the writer,[4] has regularly been confused with the former. In the genus *Prenanthes,* almost all its species differ from their relatives in *Lactuca* and *Hieracium* in possessing not only five main bundles within the ovary, but in addition one to twelve supernumerary ones. The homology of all these is not yet clear, but in some species the dorsal carpellary bundles are free from those of the corolla, in others the ventral bundle or bundles appear to be extended up into the ovary wall beyond the insertion of the trace to the basal ovule, while still others apparently possess more or less vestigial calyx bundles. Closely paralleling *Prenanthes* in ovary anatomy is the group of Sino-Himalayan high alpine plants generally referred to *Crepis* as the section *Glomeratae,* but which, on the basis of anatomy as well as the external morphology of the corollas, stigmas and achenes, should be placed in a separate genus more nearly related to *Prenanthes* than to *Crepis.*[5] A treatment of this genus is to be undertaken in the near future. In the case of both the *Glomeratae* and *Ixeris* floral anatomy has proven a valuable aid to systematic classification in groups of which preserved material of critical species would be difficult or impossible to obtain.

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[3] Minna F. Koch, *Am. Jour. Bot.,* 17: 938–952, 1930.
[4] G. L. Stebbins, Jr., *Jour. Bot.* (London), 43–51, 1937.
[5] E. B. Babcock, Essays Geobot., in honor of W. A. Setchell, pp. 9–53, Univ. Calif. Press, 1936.

A SUBSTITUTE FOR WHITE INK FOR USE ON SHELLACKED KYMOGRAPH TRACINGS

THE use of white ink for writing on shellacked kymograph paper has proved very unsatisfactory. The fact that it chips and rubs off the paper makes it hazardous to add notes to a tracing. The writer has found that titanium dioxide added to white shellac makes an excellent preparation for this purpose. The advantages are that it can be applied with an ordinary pen—a stub point held sideways serving to the best advantage; it dries rapidly and is permanent; it does not rub off on handling or brushing; it photographs well and contrast slides are excellent.

Titanium dioxide is rubbed up to a soft paste with white shellac, and then more shellac or alcohol is added to give proper consistency. This will vary with the type of pen point used and the manner of individual writing. A slow motion in writing is necessary, and only a small amount of material is applied to the point. If the point is kept clean by frequent wiping and dipping in alcohol, there will not be an accumulation of pigment on the point.

Other substances used for pigment have proved unsatisfactory. Zinc oxide does not cover well and gives a distinct bluish cast to the writing. Lead salts apparently are too heavy to flow easily.

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BOOKS RECEIVED

ALBERT, A. ADRIAN. *Modern Higher Algebra.* Pp. xiv + 319. University of Chicago Press. $4.00.

BURNET, F. M., E. V. KEOGH and DORA LUSH. *The Immunological Reactions of the Filterable Viruses.* Pp. 227–368. University of Adelaide, Australia. 10s.

FROLOV, Y. P. *Pavlov and His School; The Theory of Conditioned Reflexes.* Pp. xix + 291. 26 figures. Oxford. $4.00.

GOLDBLATT, L. A. *Collateral Readings in Inorganic Chemistry.* Pp. 225. Appleton-Century. $1.35.

GRAY, ALBERT A. *The Basis of Tissue Evolution and Pathogenesis.* Pp. xix + 92. Jackson, Son & Co., Glasgow, Scotland. 7/6.

Handbook of Scientific and Technical Societies and Institutions of the United States and Canada. Third edition. Pp. 283. National Research Council, Washington. $3.50.

HUBBLE, EDWIN. *The Observational Approach to Cosmology.* Pp. vi + 68. Illustrated. Oxford. $4.00.

MERRILL, PAUL W. *The Nature of Variable Stars.* Pp. vii + 134. Macmillan. $2.00.

TAYLOR, GRIFFITH. *Environment, Race and Migration.* Pp. xv + 483. 158 figures. University of Chicago Press. $4.00.

WEGENER, ALFRED. *La Genèse des Continents et des Océans.* Pp. viii + 236. Illustrated. Librairie Nizet et Bastard, Paris.

WOODGER, J. H. *The Axiomatic Method in Biology.* Pp. x + 174. Cambridge University Press, Macmillan. $3.75.