

determining factor in this movement. For in the cowslip the very opposite occurs. The flowers, at first pendant, offer greater facilities as far as self-pollination is concerned to the long-styled forms, but later on the flowers become erect and thus in the absence of cross-pollination the short-styled forms will have ample opportunity to be self-pollinated.

From the observations I have made on the primrose I feel convinced that it is both regularly visited and cross-pollinated by insects under favourable climatic conditions, but that like most flowers adapted to the visits of insects, it is provided with efficient means for self-pollination and these are important to a plant flowering at so early a period of the year when the visits of insects may be precarious.

ON A NEW METHOD FOR FACILITATING THE STAINING OF MICROSCOPICALLY SMALL OBJECTS,

BY V. H. BLACKMAN, M.A.

THERE is a great need for a really satisfactory method of dealing with microscopically small objects so that they can be stained by the most modern methods and handled as easily as are microtome sections fixed to a slide.

The obvious method, by *decantation*, in which the objects (small unicellular organisms, etc.) are fixed, washed and stained by successive quantities of fluid which are decanted off from the sediment of material, is objectionable, not only from its tediousness and difficulty of thorough washing, but also from the fact that it prohibits the use of the more delicate cytological stains. With these stains it is very generally necessary that a given fluid, after producing the right degree of differentiation, should be quickly and completely removed; such a rapid change of fluid is however impossible by the decantation method.

The difficulty in question could clearly be got over by fixing the objects to a slide in the manner of sections. For this purpose two methods have been devised, but both have considerable objections. One method is that of Overton (*Zeit f. wiss. Mikroskopie* Bd. vii. 1890, p. 9), in which the objects, after being brought up to absolute alcohol (if necessary, by placing them in dilute alcohol

in an atmosphere of alcohol), are fixed to the slide by means of a thin film of celloidin. The objections to this method are, that though it allows of a rapid transference from one fluid to another, yet the presence of the film, not only introduces difficulty of clearing, but also prohibits the use of certain stains which colour the celloidin too deeply.

A considerable step in advance was made by the "Stippling" method of Harper and Fairchild (Trans. Wisconsin Acad. of Sc., Vol. XII., p 479) in which the fixing fluid together with the objects is "stippled" in drops by means of a fine pipette, upon the surface of a slide prepared with Mayer's albumen fixative. The fixing fluid partly coagulates the albumen and the coagulation is completed by passing through increasing strengths of alcohol, so that the objects remain firmly fixed to the slide. This method is exceedingly ingenious, but is difficult to carry out, is applicable only to very small objects, and, as Harper states, a large proportion of the material is lost in the process. The method now to be described is applicable to both small and large objects (*e.g.* large Desmids and large teleutospores) and when carefully used all the material placed upon the slide is held fast. Historically it would appear to be a modification of the Harper and Fairchild process, but it was actually arrived at by a consideration of the condition of affairs when one melts the paraffin of a microtome section which is lying upon a layer of albumen fixative.

The material to be treated is brought up by decantation, filtration, etc., if necessary, very gradually, to some clearing fluid (xylol, cedar-wood oil, bergamot oil, &c.). Drops of the clearing fluid containing the objects are then placed upon the surface of a slide prepared with egg-albumen; either in fairly large drops or the "stippling" process can be used, according to the closeness of the objects in the fluid. The drops spread out, but the objects are retained by the albumen. When the fixing fluid has evaporated and spread out to a very thin layer, the slide is placed slightly obliquely, and absolute alcohol is allowed to flow very slowly from a pipette over the surface of the slide. The clearing fluid is washed away, but the objects are retained in position by the sticky albumen, which, being immediately coagulated by the alcohol, holds the material fast. The slide can then be treated in the same way as one to which microtome sections have been fixed. Of the clearing

¹ Harper states that the method can be made applicable to larger objects by washing away the fixative with fairly strong alcohol, but in my hands this has led to collapse of the cells.

fluids xylol is found to be rather too volatile to be easy of use, a mixture of equal parts of xylol and cedar-wood oil works, perhaps, most easily. If cedar-wood oil is used, the objects can very conveniently be brought up to this fluid by placing them in a 10% solution of cedar-oil in absolute alcohol, over calcium chloride in a dessicator. The spreading out of the drops of cedar-wood oil on the slide should be accelerated by warming on the paraffin oven.

The method is, of course, a much longer one than that of Harper and Fairchild, as the objects have to be brought up to a clearing fluid before they can be placed on the slide, but it has the advantages of being easily applicable to both large and small objects and of involving no loss of material in the process of fixing to the slide. Moreover, the method can obviously be applied to material which has been fixed in any way. It is useful, among other purposes, in making slides of *plankton* material, and even with ready stained material which only requires mounting in balsam, I have found it convenient to mount on a thin layer of albumen; by this means one avoids the shifting of the objects likely to be caused by the addition of balsam or of the cover-slip.

ON DESCRIPTIONS OF VASCULAR STRUCTURES.

IN describing the course of the vascular tissues in the stem, it is more convenient in some cases to trace them in the acropetal direction, in other cases in the reverse order.

De Bary,¹ speaking of "common bundles" (*i. e.* those belonging to stem and leaf), writes as follows. "Their course in the stem is most clearly understood by following them from their point of exit in a basipetal direction, that is downwards. The description of their course in this direction also corresponds best to the facts, inasmuch as at least in most cases the development of the common bundles begins at the point of exit, and proceeds on the one hand towards the leaf, and on the other downwards in the stem." For example one may speak of a leaf-trace as passing² through the cortex of the stem into the bundle-ring and then downwards through so many internodes before fusing with the leaf-trace of a certain

¹ De Bary: *Comparative Anatomy*, English Edition, p. 234.

² The metaphorical use of words signifying motion, in the same manner as they would be employed for describing the course of a road, avoids a lengthy periphrasis.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.