A METHOD FOR MAKING ACETO-CARMIN SMEARS PERMANENT

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ABSTRACT.—**Anthers are collected and placed in a solution of 1 part acetic acid to 3 parts of absolute alcohol. The contents of the anther are squeezed out on a slide in a drop of Belling's iron-aceto-carmin solution and a cover glass placed over the drop. Care should be taken to remove all anther walls and flower parts. Heat the slide over an alcohol flame for a second, repeating 4 or 5 times. Place the slide in a petri dish filled with a 10% solution of acetic acid. When the cover glass has risen away from the slide gently remove the cover glass and place in a Coplin jar containing equal parts of alcohol and acetic acid. Likewise, place the slide in this solution. Run both cover and slide thru the following solutions: 1 part acetic acid to 3 parts absolute alcohol, 1 part acetic acid to 9 parts absolute alcohol, absolute alcohol and finally equal parts of absolute alcohol and xylol. Recombine the cover and slide in xylol-balsam directly from this solution.**

With the increasing use of Belling's iron-aceto-carmin method for making chromosome counts and its partial displacement of the tedious but permanent paraffin method has come the need for a record of such counts in the form of permanent preparations. One advantage of the aceto-carmin method is its speed; one disadvantage, a lack of permanency in the preparations. Altho several methods of making such preparations permanent have been suggested[1] the author has had more consistent results with the method to be outlined below.

Material is collected and placed immediately in a bottle containing 1 part of glacial acetic acid to 3 parts of absolute alcohol. Altho maize sporocytes have been kept in this killer for from several days to several weeks, the very best preparations were obtained from material that had been in killer from 12–24 hours. Over a short period of time, at least, this killer decidedly improves the appearance and stainability of the chromosomes in aceto-carmin preparations.

[1]Belling, J. The iron-aceto-carmin method of fixing and staining chromosomes. Biol. Bull., **50**, 160–162. 1926.

Heitz, E. Der Nachweis der Chromosomen. Vergleichende Studien über ihre Zahl, Grösse und Form im Pflanzenreich I. Zeit. f. Bot. **18**, 625–681. 1926.

Longley, A. E. Supernumerary chromosomes in Zea mays. J. Agr. Res., **35**, 769–784. 1927.

For longer storage the material can be transferred directly from the killer to 70% alcohol, and aceto-carmin preparations can then be made at any later time (Fig. 1). Anthers can be transferred from the killer or 70% alcohol to a small drop of iron-aceto-carmin[2] solution. The contents of the anther should be carefully squeezed or teased out into this drop. At this point it is important to mention that all anther walls or flower parts, other than the sporocytes themselves, should be removed so that the cover-glass, when placed over the drop, will come into direct contact with the sporocytes. After the cover-glass has been gently placed over the material, the slide is

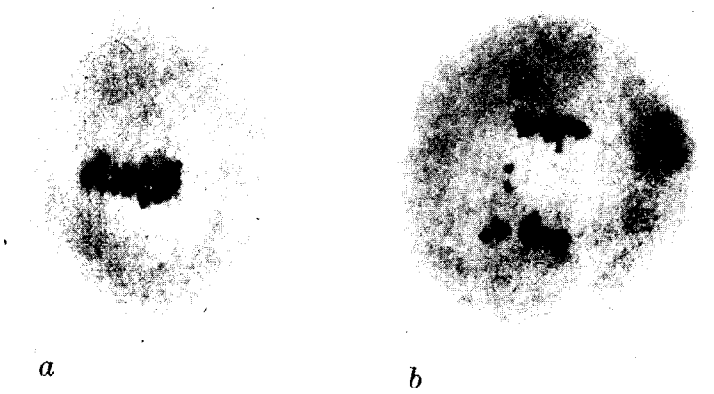

FIG. 1. Photomicrographs of microsporocytes of *Zea mays* from permanently mounted aceto-carmine smears: *a*, metaphase of first meiotic mitosis; *b*, anaphase of first meiotic mitosis.

held over an alcohol flame for about one second. This should be repeated 4 or 5 times until the cells are somewhat spread and flattened. Heating greatly increases the contrast between chromosomes and cytoplasm and brings about an adhesion of the sporocytes to the slide or cover-glass. Likewise, material that has been in killer or in alcohol for some time is very much improved by heating. The solution must not be allowed to boil! Experience will be required to determine the amount of heat necessary to give the best results.

Where a great many slides are to be studied during a season, and where it is neither necessary nor desirable to render permanent all

[2]The writer has employed certified carmine (NCa 2) in this technic.

the slides made, it is advisable to examine each slide soon after making in order that it may be placed in one of the following categories: (1) to be discarded after examination; (2) to be made permanent immediately; (3) to be ringed, so that the slide may darken or await further examination.

The next step depends upon whether the slide is to be made permanent immediately after examination or whether it has been ringed. If it is to be run up immediately, the immersion oil should be removed from the upper surface of the cover-glass. This should be done *very carefully*. The cover-glass must not be allowed to shift or the sporocytes will be pushed loose and will not stick to the glass. The slide is then placed in a petri dish filled with a 10% solution of acetic acid. The cover-glass will gradually rise somewhat from the slide, carrying some of the sporocytes with it. The cover-glass should be gently pushed over the edge of the slide so that it can be grasped with forceps. The cover-glass must not be pushed before enough solution has run between the slide and cover-glass to allow the latter to float freely. The sporocytes will be found to have stuck to both the slide and the cover-glass; consequently both must be handled in the solutions that follow. Carefully transfer the slide to a Coplin jar containing equal parts of acetic acid and absolute alcohol; likewise, transfer the cover-glass into the same solution. Then pass both thru the following solutions, a few minutes in each solution being sufficient: 1 part acetic acid to 3 parts absolute alcohol, 1 part acetic acid to 9 parts absolute alcohol, absolute alcohol, and finally equal parts of absolute alcohol and xylol. The slide and cover-glass are recombined directly from this solution in xylol-balsam. A pure xylol solution must not be used on fresh slides as it will cause a distortion of the sporocytes. To obtain the best results, the solutions should be frequently renewed. The slide is removed from the alcohol-xylol solution and a drop of fairly thick xylol-balsam is placed over the sporocyte region. The cover-glass is then placed face down on the drop of balsam. This last operation should be done rapidly to prevent absorption of moisture and consequent clouding of the medium.

If a slide is not to be run up immediately it should be ringed with some brittle, non-sticky medium such as paraffin. The following mixture[3] is preferred since it forms a perfect seal and is quite easily removed. Heat equal parts by weight of paraffin and gum mastic and stir thoroly. Allow to cool. This mixture can be applied to the edges of the cover by means of a heated wire. Bend the end of a

[3]Used for some time in the laboratory of Prof. Otto Rosenberg at Stockholm.

piece of wire so that it can be placed parallel to the long edge of the cover-glass. Heat this end of the wire in a flame and place in the paraffin-mastic mixture. Enough of the mixture will melt and remain on the wire so that it can be carried to the edge of the cover-glass where it will immediately solidify. The slide will keep for some time thus sealed but will gradually become dark. Likewise, in this strong acetic acid solution the chromosomes will show signs of disorganization. If a weaker solution of acetic acid is used this disintegration apparently does not proceed so rapidly (Randolph). If at a later time it is desired to run the slide into balsam, the seal must be removed. This, of course, must be done very gently so that the cover-glass will not move. The paraffin-mastic mixture will be found to chip away readily. First remove the seal carefully from 3 sides. When this has been done a weak spring clip should be placed over the cover and slide to keep the cover from moving when the seal is being removed from the fourth side. When the seal has been sufficiently removed so that the cover will float free in a solution, proceed as has been directed.

If the slide has become too dark, the carmin stain can be removed by allowing a solution of acetic acid to run under the cover. The slide is then held over an alcohol flame and gently heated until the slide is sufficiently destained.