An Eclectic Method for Preparing Chromosome Squashes

By THOMAS P. EVANS

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A large number of secondary-school biology textbooks and accompanying laboratory activities have been revised within the past two years. Nevertheless, most still suggest the use of onion root tips and acetocarmine or aceto-orcein stain for preparing chromosome squashes. They suggest these materials in spite of the fact that their preparation and use are time-consuming and the results are poor.

The purpose of this article is to describe a quick, simple, foolproof method for preparing chromosome squashes. The method is eclectic in that it was developed by trying and combining the best ideas from a number of methods (Carlock and Moore, 1970; Hilbert, 1969; Lawson and Paulson, 1960; Parker, 1968; Stopyra, 1969). It uses Wandering Jew (*Tradescantia* sp.) and toluidine blue O stain. It has the advantage over other methods in that squashes of excellent quality can be accomplished by most students in about five minutes. A second purpose is to suggest alternate methods for making the squashes into permanent slides.

Materials

The materials required for making the squashes are as follows: freshly cut Wandering Jew, 0.5% aqueous solution of toluidine blue O stain, 1 N hydrochloric acid, razor blades, forceps, bunsen burners, paper towels, microscopes, glass slides and cover slips. Additional materials are required for preparing permanent mounts; these include absolute ethyl alcohol, dry ice, and a commercially available

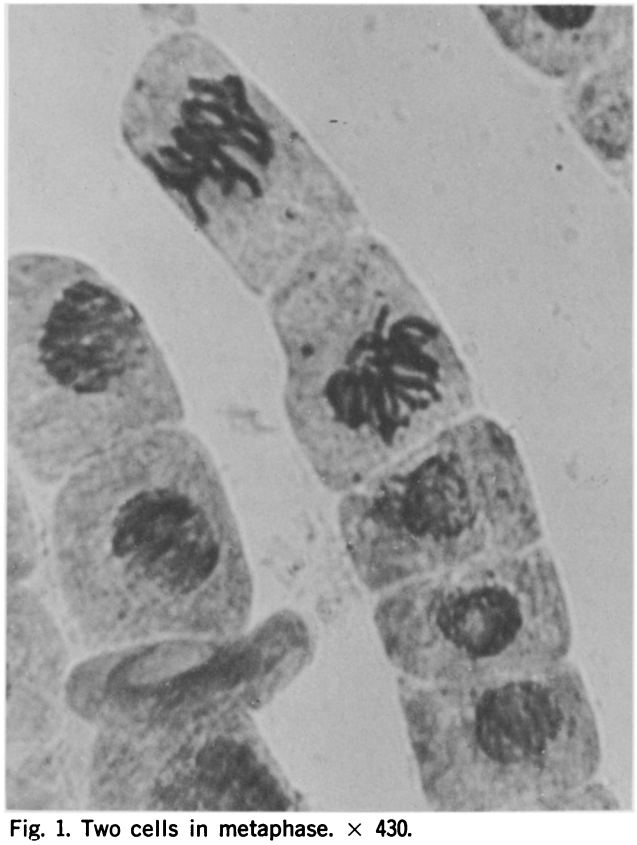

Fig. 1. Two cells in metaphase. × 430.

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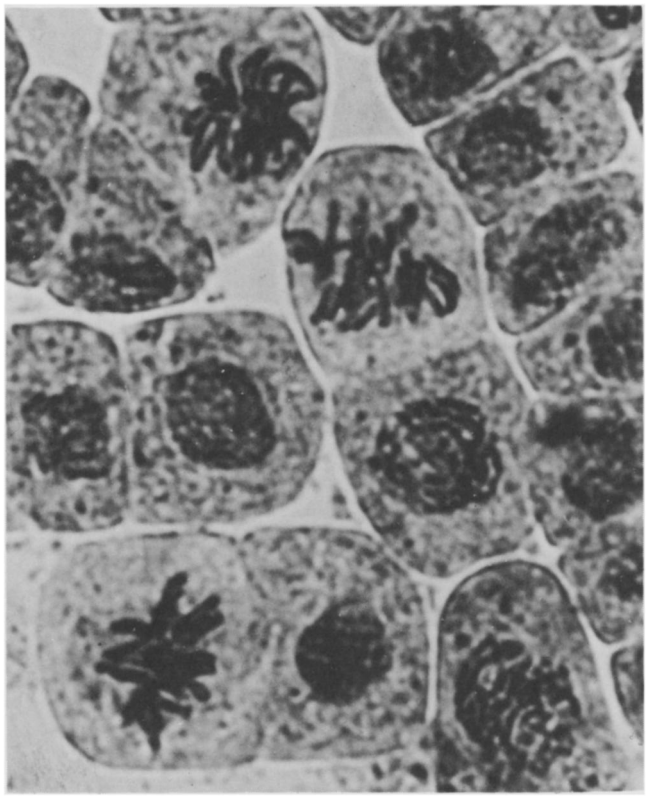

Fig. 2. Cell in anaphase, with spindle fibers visible. × 430.

mounting medium such as Permount or Diaphane.

Root tips are obtained by removing the lower leaves of 10-to-13-cm cuttings of Wandering Jew and placing the cuttings in a beaker of water. Roots 1 to 3 cm long appear in four days. The use of Wandering Jew has several advantages over the use of onions: (i) Root growth in Wandering Jew is more predictable, since onions are usually treated to inhibit root growth. (ii) Wandering Jew does not have to be supported with toothpicks. (iii) Unlike onion root tips, large numbers of mitotic figures are present in Wandering Jew root tips at nearly any hour of the day; thus, the necessity of cutting and preserving roots at certain hours is eliminated. (iv) Since students can remove roots from Wandering Jew and find mitotic figures at any hour of the day, the problem that students often have in associating preserved roots and mitotic figures with the living organism should be reduced. (v) Wandering Jew is fast-growing, and sufficient quantities can be grown in the laboratory with little effort.

The stain is prepared by dissolving 0.5 gg of toluidine blue O stain in 100 ml of water. The advantages of preparing and using toluidine blue O stain are fairly obvious. It does not require heating, filtering, or the addition of glacial acetic acid and ferric hydrate. An old scalpel or nail is not required for stirring and adding additional iron to the stain. And toluidine blue O stain can be stored indefinitely in glass, stoppered bottles at room temperature.

Standard reagent grade hydrochloric acid is 12 N. Prepare 1 l of 1 N acid by adding 83 ml of concentrated acid to 917 ml of water.

Procedure

The procedure for preparing chromosome squashes is carried out completely on a slide. The steps are as follows:

1. After the roots reach 1 to 3 cm in length, remove an entire root from a cutting and place the root on a clean slide. (Actually, good results have been obtained with roots ranging up to 5 cm in length.) Return the cutting containing additional roots to the beaker of water. Removing the entire root rather than just cutting off the tip prevents other students from obtaining a piece of root without a root tip.
2. Cut off the root tip 1 to 2 ml behind the root cap, discarding the upper portion.
3. Place three drops of 1 N hydrochloric acid on the root tip for approximately 1 min. During this time, heat the slide gently over a low flame until the heat becomes uncomfortable. Repeat the heating two or three times, but do not allow the liquid to boil. The root tips appear fuzzy at the end of the treatment.
4. Blot off the acid with a strip of paper towel and cover the root tip with two or three drops of stain. Warm the slide for approximately one minute as in the acid treatment.
5. Blot off the excess stain and add a drop of fresh stain. Apply a cover slip. Place the slide in a folded

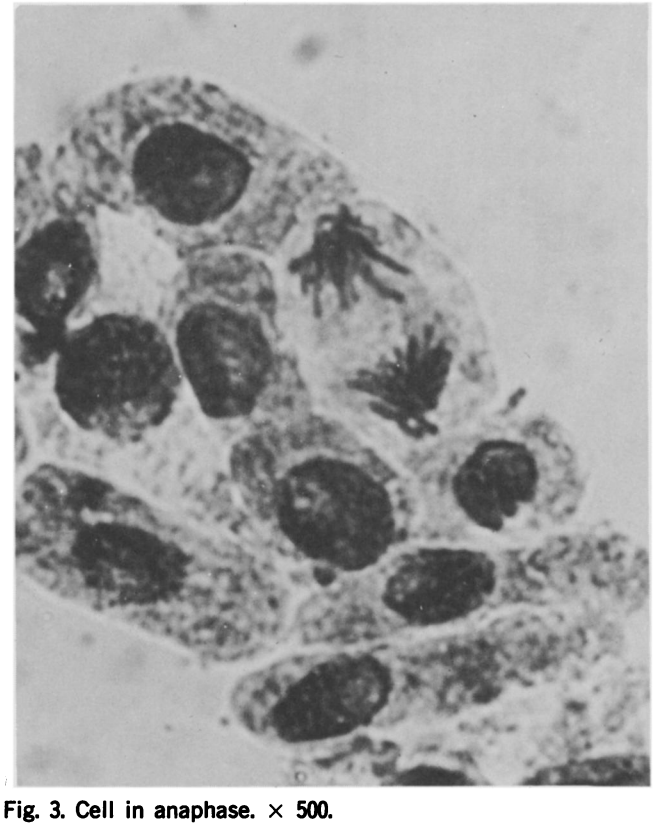

Fig. 3. Cell in anaphase. × 500.

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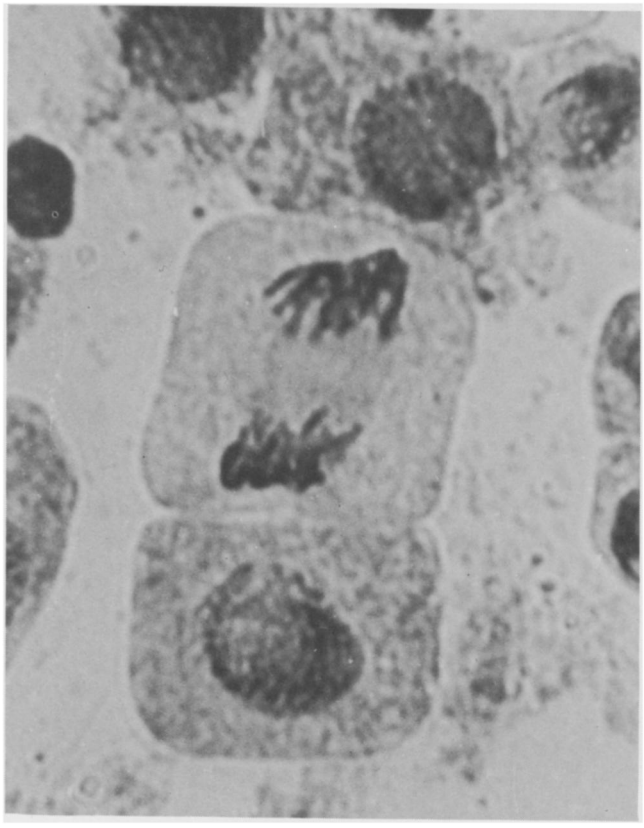

Fig. 4. Three cells in metaphase; polar view at bottom. × 500.

paper towel on a hard surface and squash firmly with your thumb. Or squashing can be accomplished with a pencil: while the slide is on a hard surface, place the eraser end on the cover slip directly above the root tip and press firmly straight down.

6. Clean the stain from the slide and the cover slip and examine under low power for embryonic cells. They appear square (see fig.). Once the cells are located, switch to high power. If cells are stained too darkly, the stain can be diluted by placing one or two drops of water at one edge of the cover slip and a paper towel at the opposite edge. Repeat this until the stained portions of the cells reach the desired contrast.

7. A light coat of fingernail polish or paraffin around the edges of the cover slip will prevent the squash from drying, for several hours. It should be noted, however, that this sealing will interfere with making the squash into a permanent mount.

Permanent Slides

If squashes of particularly excellent quality are obtained, they can be turned into permanent slides in the following manner:

1. Place the slide on a piece of dry ice for about one minute. As the squash freezes, pry off the cover slip with a razor blade. An alternate method for removing the cover slip involves immersing the slide into a 1:1 solution of acetic alcohol (Stopyra, 1969); however, this method has proven to be only partly successful, because the squash frequently washes off of the slide or remains partly attached to the cover slip.

2. After removing the cover slip, immerse the slide containing the squash for two or three minutes in absolute ethyl alcohol.

3. Change the alcohol and allow the slide to remain immersed for an additional two or three minutes. (The use of certain mounting media requires an additional bath, for three minutes, in toluene; see manufacturer's directions.)

4. Remove the slide from the alcohol and, before the squash dries, place a drop of mounting medium on the squash. Cover the squash with a clean cover slip.

Regardless of the mounting medium used, allow the slides to remain flat until the medium hardens; 24 hours is usually long enough. Once the medium hardens, clean the excess medium from the slides and store the slides on their edges in a slide box.

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Hormonal Control of Insects

The search for a way of controlling insects without resorting to methods harmful to the environment will be enlarged under a National Science Foundation grant of $70,000 to a Northwestern University biologist.

The grant, to Lawrence I. Gilbert, will support a two-year study aimed at providing scientists with a piece of the puzzle, the whole answer to which may permit control of the growth of certain insects by treating them with synthetic versions of two hormones known to regulate their development.

As substitutes for DDT and other chemical means for deterring the proliferation of insect pests, hormones are believed to have two highly advantageous features. Whereas insects tend to develop an immunity to chemical pesticides, it has been suggested that they would not be able to form resistance to their own hormones, Gilbert said. In addition, he added, there is as yet no evidence that the two insect hormones to be studied have harmful effects on vertebrates.

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